

SHORT COMMUNICATIONS

SPECTROPHOTOMETRIC STUDIES ON THE INTERACTION BETWEEN LACCAIC ACID AND DNA

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LACCAIC acid is the water soluble lac-dye obtained from the wash water in the lac factory while converting sticklac (lac encrustation scraped from twigs) to the lac resin of commerce. The structure of laccaic acid was studied by Venkataraman and co-workers¹ who showed it to be anthraquinone derivative.

Recent work on laccaic acid is interesting. Chakravarty *et al*^{2,3} studied the effect of laccaic acid on the hepatic biochemistry of rat. A significant reduction in the RNA and DNA contents was observed at extremely high doses of laccaic acid². Total lipid, total sterol, bound sterol contents of the liver and also lipid/protein ratio were found to reduce at high doses². Studies also indicated that lac-dye inhibits the activities of mitochondrial transaminases at very high doses³. It was concluded that at very high doses, lac dye interferes with the normal physiological functioning of the liver³.

It appears that laccaic acid belongs to that class of compounds viz acridines (proflavine, acridine orange)⁴ and anthracyclines (nogalamycin, daunomycin)⁴ etc where biological manifestations arise from their ability to get bound with DNA⁴. It was therefore interesting to study the interaction between laccaic acid and DNA. The present communication reports the results of a brief study on the interaction between laccaic acid and DNA.

Highly polymerized salmon testes DNA (Sigma Chemical Co., USA) was used in this study. Concentrations of the DNA solutions were determined spectrophotometrically in terms of the phosphate residues, assuming the molar extinction coefficient to be of the order of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ at 258 nm.

Laccaic acid was prepared by the method of Ghosh and Sengupta⁵. The molecular weight of laccaic acid was taken as 485. Laccaic acid showed an absorption maximum at 490 nm in water. Its molar extinction coefficient in water was $5964 \text{ M}^{-1} \text{ cm}^{-1}$.

Spectrophotometric measurements were carried out with a Beckman DBG T spectrophotometer. The solutions of DNA-dye complexes were prepared following Das Gupta *et al*⁶ (which is a modification of the method of Peacocke and Skerrett⁷) at different DNA phosphate (P) to dye (D) ratios (P/D). The final concentration of laccaic acid was kept constant in all the complexes. The solvent used was 0.001 M NaCl and the pH was about 6.7.

Different binding parameters viz α the bound fraction, r the number of dye molecules bound per nucleotide and C the free dye concentration were determined following the method of Peacocke and Skerrett⁷ and were plotted according to the Scatchard binding equation

$$r/C = K(n - r) \quad (1)$$

where K is the apparent binding constant and n is the number of binding sites available per nucleotide⁷.

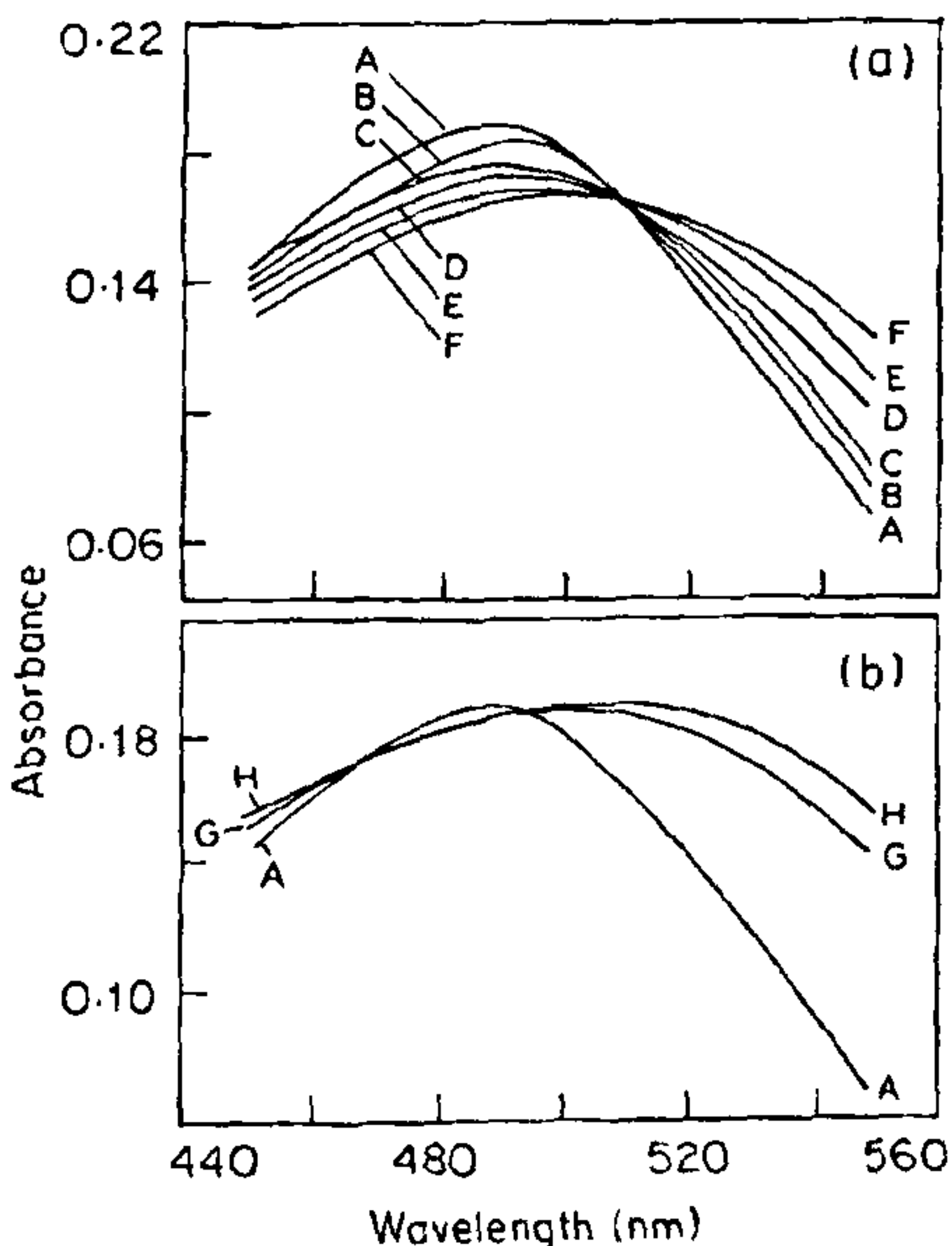


Figure 1a,b. Changes in the absorption spectrum of laccaic acid due to the progressive binding of DNA. Lac-dye concentration = $3.5 \times 10^{-5} \text{ M}$. Value of P/D: (A) Free dye, (B) 2.3, (C) 6.9, (D) 11.4, (E) 18.3, (F) 23, (G) 34.3, (H) 45.7.

Figure 1a shows the changes in the absorption spectra of the lac-dye due to progressive addition of DNA (increasing P/D ratios). The spectral changes involved essentially a progressive red shift and hypochromicity in the complexes up to P/D = 23. Further addition of DNA i.e. for complexes P/D > 23 resulted in an increase in the absorbance (figure 1b). The maximum red shift was obtained approximately 20 nm (from 490 to 510 nm). An isosbestic point was obtained at about 507 nm for the spectra of DNA-dye complexes for P/D values up to 23.

Figure 2 shows the variation of the absorbance at 490 nm of the DNA-dye complexes with the rise of P/D. For low P/D values, an initial decrease in absorbance was observed; it reached a minimum value and then increased again with further rise in P/D.

Different binding parameters r and r/C were calculated from the changes in the absorbance at 490 nm. Figure 3 shows the plot of r/C vs r , which represents the Scatchard equation of binding (equation 1). The number of strong binding sites available per nucleotide (n) and the association constant (K) were determined from the plot and the values were 0.064 and $8.3 \times 10^{-5} \text{ M}^{-1}$ respectively.

The red shift observed in the present study (figure 1) suggests that the dye molecules are localized within the less polar region of the DNA polymer as has been attributed in the cases of nogalamycin⁸, proflavine and other acridine dyes⁷. The hyperchromicity observed at high P/D values was due to the intercalated monomers, as stated by earlier workers^{9,10} in the study on binding of acridine orange with DNA.

The linearity of the Scatchard plot obtained in the present study indicated that the interaction of

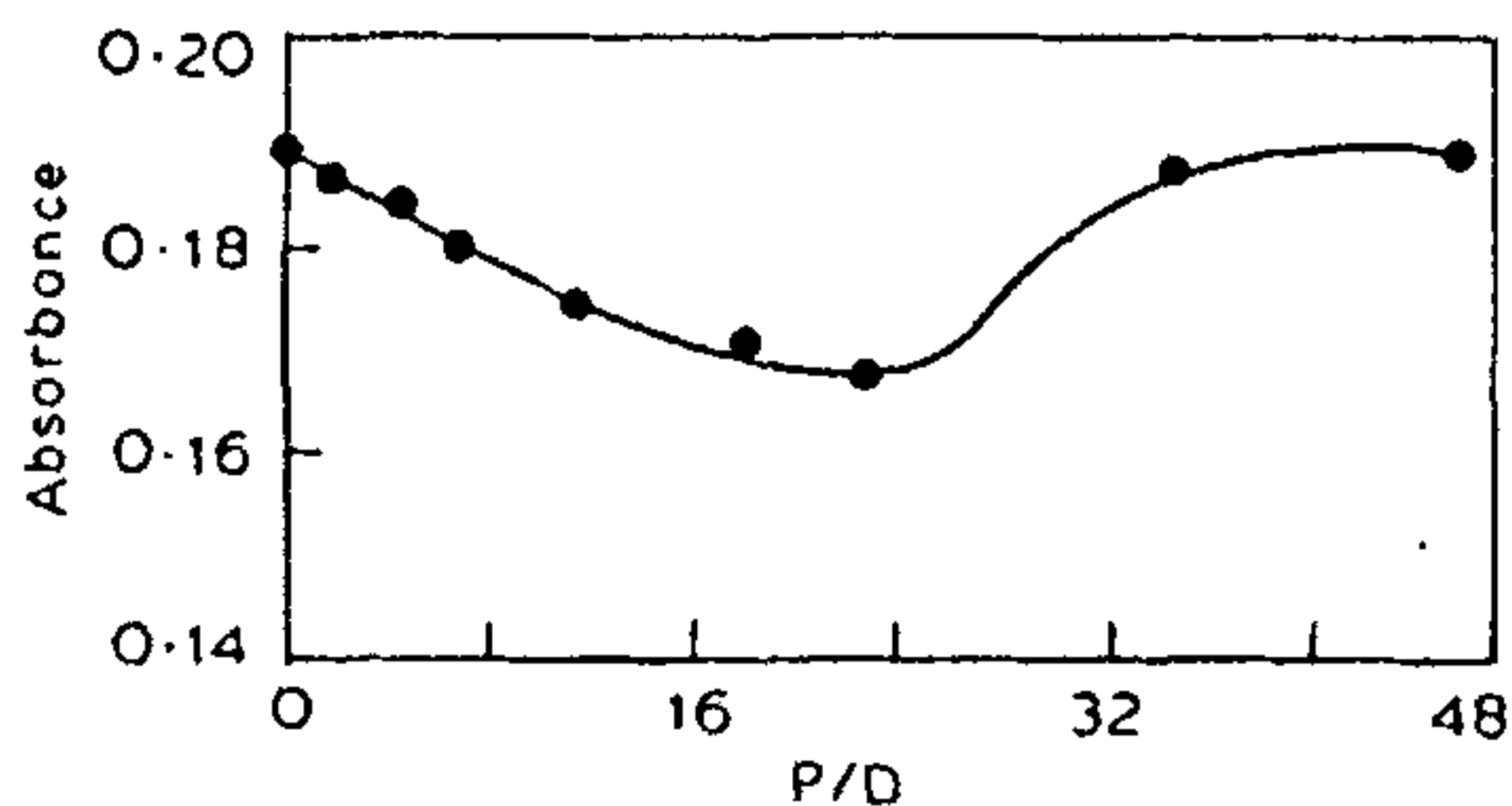


Figure 2. Variation in the absorbance of laccaic acid at different P/D values at 490 nm.

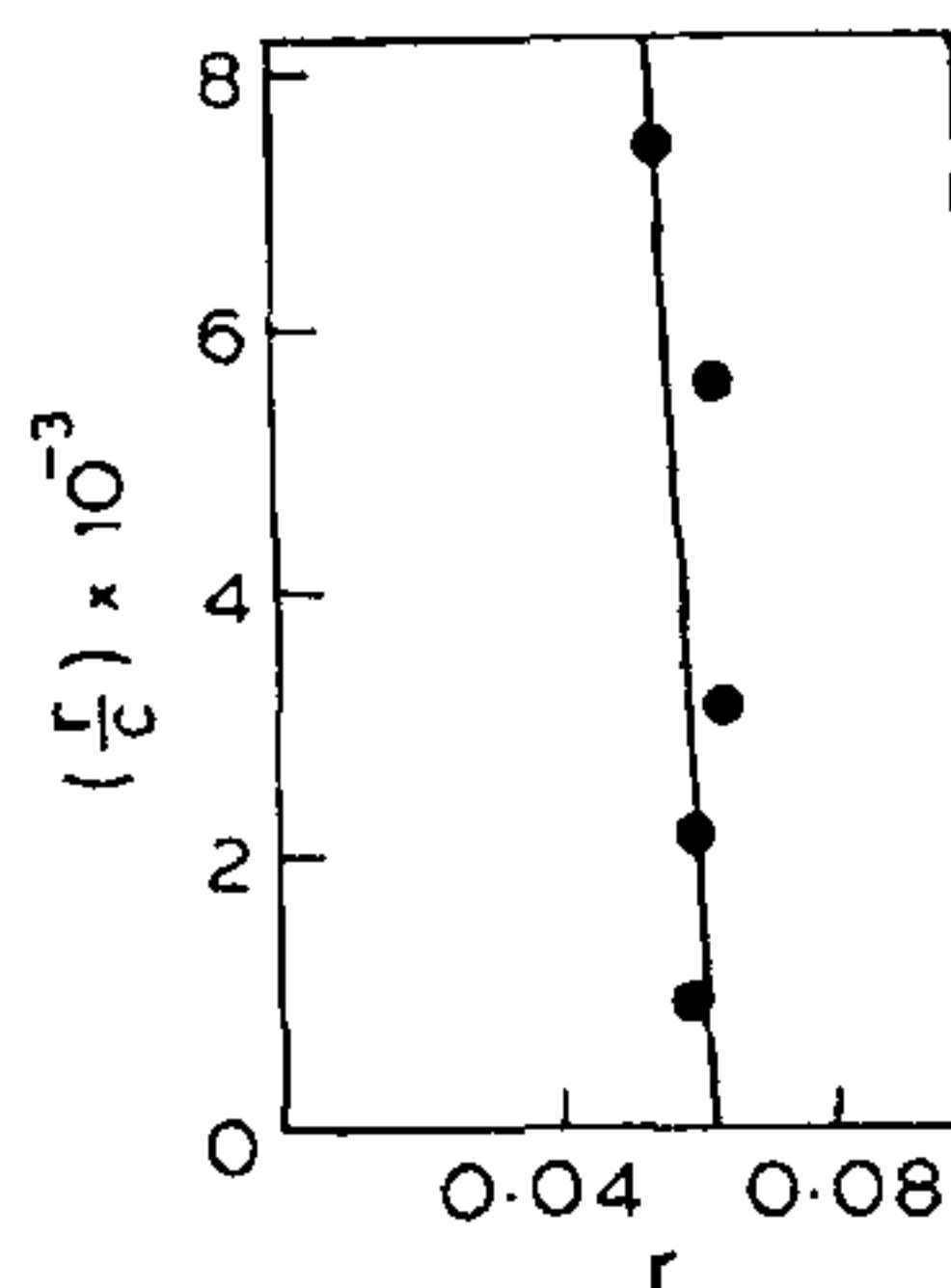


Figure 3. Scatchard plot for the binding of laccaic acid with DNA.

laccaic acid with DNA is unimodal in nature⁷. The number of available strong binding sites per nucleotide (n) for laccaic acid has been found to be low (0.064) in the present study indicating less binding affinity of the lac-dye with DNA. For actinomycin C₃, a similar low value of n (0.08) was obtained (at 0.01 M Na⁺) by Müller and Crothers¹¹. Estensen *et al*¹² also obtained a low value of n (0.025) for DNA-quinine complex (at 5×10^{-3} M tris-HCl buffer).

The present results indicate that laccaic acid undergoes binding with DNA.

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PRELIMINARY STUDIES ON THE ANTI-DIABETIC EFFECTS OF CABBAGE (*BRASSICA VAR CAPITATA* L.) OIL ON STREPTOZOTOCIN DIABETIC RATS

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CABBAGE contains S-methyl cysteine sulphoxide¹ (SMCS) and it is converted to dimethyl disulphide on crushing and extraction^{2,3}. Itokawa *et al*⁴ showed that SMCS and S-allyl cysteine sulphoxide (SACS), the precursors of the disulphide containing oils of cabbage and garlic respectively, are antihypercholesterolemic in action. Farva *et al*⁵ showed that garlic oil has definite antidiabetic action. In the present communication, the diabetic effects of cabbage oil as compared to those of insulin in streptozotocin diabetic rats are studied.

Cabbage oil was prepared from a fresh sample of the vegetable which was sliced, dried and soaked in diethyl ether for two days. The decanted solution was distilled at 40°C and the oil left was used. White albino rats weighing 100–150 g were made diabetic by intravenous injection of streptozotocin (40 mg/kg)⁶ in citrate buffer with pH 4.5. Fasting blood sugar of the rats was estimated after a week by the method of Asatoor and King⁷. Rats with blood sugar above 180 mg/100 ml were used. Plain insulin (40 units/ml) was used to study the effects of insulin in diabetic rats for a comparison of the effects of cabbage oil. Six normal rats were used to get normal values. Diabetic rats were divided into four groups of six each. All the rats were given rat food supplied by Pfizer (Kaduna, Nigeria). The particulars of the treatment were as follows for groups 3–5: (i) Normal rats for normal control values; (ii) diabetic rats for untreated control values; (iii) diabetic rats

injected daily with insulin 10 units/kg; (iv) diabetic rats injected daily with insulin 5 units/kg; (v) diabetic rats orally administered daily with cabbage oil 100 mg/kg.

Insulin was diluted in normal saline in the ratio 1:3 and 0.1 ml and 0.05 ml/100 g body weight were injected to the corresponding groups subcutaneously every day for a month. Cabbage oil was made into a suspension in normal saline and it was fed intragastrically by a stomach tube (dose 100 mg/kg) for the same period to the last group. After one month the rats were again weighed and their fasting blood sugar estimated. They were then sacrificed by decapitation and their blood, liver and kidneys were collected for various estimations. In serum and tissues, cholesterol by the method of Zlatkis *et al*⁸ and triglycerides by the method of Lambert and Neish⁹ were estimated. Total lipids in tissues were determined by a gravimetric method¹⁰. All the values were analysed statistically based on student's *t* test. A known antidiabetic agent garlic oil⁵ composed of diallyl disulphide was run side by side with cabbage oil on alumina coated with thin layer chromatography (TLC) plates using hexane:diethyl ether: glacial acetic acid (70:30:1, v/v) system for 2 hr. The plate was then dried and sprayed with sodium nitroprusside reagent to locate the organic sulphides. Purple spots appeared and their *R_f* values were measured.

On treatment with insulin and cabbage oil, blood sugar, serum and liver cholesterol, serum, liver and kidney triglycerides and total liver lipids reduced significantly. As shown in table 1, some parameters were brought to near normal and the effects of the oil were quite comparable to those of a high dose of insulin. As insulin completely prevented a weight loss and increased the weight, cabbage oil could only reduce the weight loss to half.

In TLC cabbage and garlic oils showed only single spots of sulphides with *R_f* values 0.65 and 0.66 respectively. The sulphur compound present in garlic oil is diallyl disulphide⁵ and that detected in cabbage oil could be dimethyl disulphide as reported earlier³. Cysteine sulphoxide derivatives present in cabbage, garlic and onion are converted to corresponding disulphide oxide and then to disulphides (dimethyl disulphide from cabbage and diallyl disulphide from garlic) on crushing and extraction as a result of the action of allinase². The antidiabetic action of garlic oil is well established⁵. On TLC cabbage oil and garlic oil showed spots of organic sulphides with very close *R_f* values. In controlling diabetic condition cabbage oil is as effective as a