phenol was made to react with nitric acid, vanillin, aniline and sulphanilic acid respectively to get coloured compounds. We report here a new colorimetric procedure for the determination of carbofuran and bendiocarb using *p*-nitroaniline as a coupling reagent in place of sulphanilic acid.

Reagents

(a) (i) Carbofuran and bendiocarb: Analytical and technical grade samples, supplied by Rallis India Ltd., Bangalore, were employed.
(ii) Standard carbofuran and bendiocarb solution: 100 μg/ml each in methanol
(b) Sodium nitrate, 0.3% (w/v) aqueous
(c) Sodium hydroxide, 2% (w/v) aqueous
(d) *p*-Nitroaniline solution, 0.2% (w/v) freshly prepared in 1 N HCl.

Procedure

Aliquots of carbofuran solution (0, 1, 2, 3, 4, 5, 6, 7 and 8 ml) were introduced in 50 ml standard flasks. To each one of these 10 ml of sodium hydroxide, 5 ml of sodium nitrite and 5 ml of *p*-nitroaniline were added. The solutions were made up to the mark with distilled methanol. The red-coloured compound had a maximum absorption at 520 nm and remained stable for nearly 24 hr. Absorbance values were recorded using an Elico spectrocolorimeter. The plot between concentration *vs* absorbance was linear over the composition studied.

Carbofuran in technical grade samples was determined with the aid of caliberation plot using the aforesaid procedure. Bendiocarb was also determined by employing this method. The red-coloured compound formed here had a maximum absorption at 520 nm and remained stable for about 6 hr.

The data relating to the analysis of technical grade samples of carbofuran and bendiocarb are presented in tables 1 and 2. The results point out that the carbamates can be determined with a relative error of 1%. The minimum amount determined by this method is 2 ppm. The results suggest that the method can be extended for the analysis of the pesticides in field water samples.

One of the authors (CVR) is grateful to M/s. Rallis India Ltd., Bangalore, for supplying analytical and technical grade samples of the carbamates.

4 October 1983; Revised 21 May 1984

Table 1 *Analysis of 75% technical grade sample of carbofuran*

Sample	Conc. of the sample in ppm	Carbofuran found (in ppm)	Carbofuran %
1	2.3	2.3	75.0
2	4.6	4.7	74.5
3	6.9	7.0	75.0
4	9.2	9.3	75.3
5	11.5	11.7	74.8
		Av. 74.9	
		Std. dev. 0.3	

Table 2 *Analysis of 96% technical grade sample of bendiocarb*

Sample	Conc. of the sample in ppm	Bendiocarb found (in ppm)	Bendiocarb %
1	2.9	2.9	95.3
2	5.8	5.8	96.0
3	8.7	8.7	95.8
4	11.6	11.6	96.0
5	14.5	14.5	95.3
			Av. 95.7
			Std. dev. 0.4

1. Tolein, J. S., *J. Occup. Med.*, 1970, **12,** 16.
2. Handa, K. Swadesh, *J. Assoc. Off. Anal. Chem.*, 1980, **63,** 200.
3. Handa, K. Swadesh and Dikshit K. Anand, *J. Assoc. Off. Anal. Chem.*, 1978, **61,** 1513.
4. Rangaswamy, J. R., Vijaya Sankar, Y. N. and Prakash, S. R., *J. Assoc. Off. Anal. Chem.*, 1976, **59,** 1276.
5. Mithyantha, M. S. and Perur, N. G., *Curr. Sci.*, 1974, **43,** 578.

MECHANISM OF THE INHIBITION OF THE BINDING OF DEOXYADENYLIC ACID TO DEOXYADENYLATE ANTIBODIES BY PYRIDINE

S. HONNAIAH and T. M. JACOB

Department of Biochemistry and ICMR Centre for Cell Biology & Genetics, Indian Institute of Science, Bangalore 560012, India.

PURIFICATION of proteins by affinity chromatography[1] utilizes their interaction with specific ligands. The methods generally used for the dissociation of the

protein-ligand complexes are, low or high pH, high ionic strength and use of reagents like urea, thiourea or guanidium hydrochloride. Humayun and Jacob showed[2] that pyridine at low concentrations can be used efficiently for this purpose without affecting the biological activity of the protein. The protein-ligand systems they studied were binding of N^6-Isopentenyl adenosine (i^6A) to anti-i^6A and dpA to anti-dpA. Jayabaskaran *et al* have shown[3] that the inhibition of the binding of anti-i^6A to i^6A is of non-competitive type and have suggested that the inhibitory effect may be due to conformational changes in the protein. If this mechanism is operating, pyridine can be expected to be a reagent of general applicability for dissociation of protein-ligand complexes. It has been found that pyridine is a good inhibitor of the binding of anti-dpC to dpC[4], anti-dpG to dpG[5], anti-dpT to dpT, anti-dpA to dpA[2], anti-dpApT to dpApT[6] and anti-dpTpA to dpTpA; but, among these systems it is comparatively more efficient in the case of anti-dpA binding to dpA. The studies reported in this communication is an attempt to understand why pyridine inhibits this system more efficiently.

Nitrocellulose membrane filters (MDI, 0.45 μ) were from Advanced Microdevices, Ambala, India. ^{3}H-dpA (Sp. Activity 700 cpm/pmole) was prepared from dpA by tritium labelling at Bhabha Atomic Research Centre, Bombay, India, and purified by paper chromatography in isopropanol-ammonia-water (7:1:2, v/v/v). Pyridine was purified by refluxing it with *p*-toluene sulfonyl chloride and subsequent fractional distillation. Protein was estimated by Lowry technique[7]. Tris-buffered saline (TBS) had the composition of 0.14 M NaCl, 0.01 M Tris-HCl (pH 7.3) and 0.02% sodium azide. Radioactivity was monitored in Beckman liquid scintillation counter, using 0.5% PPO in toluene as the medium.

Limulus polyphemous hemocyanin-deoxyadenylic acid (LPH-dpA) conjugate was prepared by coupling dpA to LPH by the carbodiimide method[8]. The conjugate had approximately 900 molecules of dpA

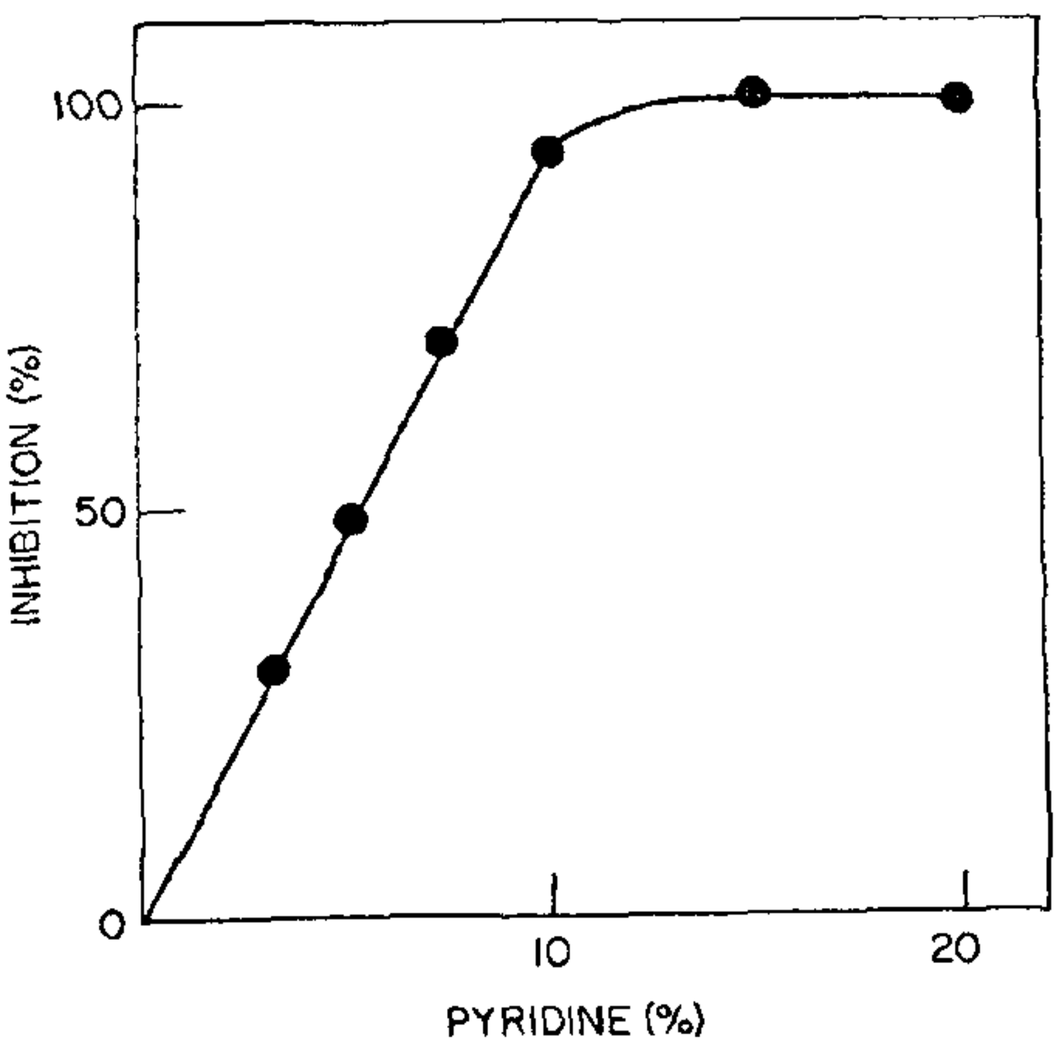

Figure 1. Pyridine inhibition of ^{3}H-dpA binding to dpA antibodies. The reaction mixture contained purified dpA antibodies (24 μg), ^{3}H-dpA (22,585 cpm), and TBS with/without pyridine in a total volume of 0.3 ml. The antibodies were added last. Incubation was for 10 min at 0°C. The reaction mixture was filtered through 25 mm MDI nitrocellulose filters (0.45 μ) under gentle suction and washed with TBS (2 × 5 ml). The filters were dried in vials at 100°C for 20 min, cooled, 0.5% PPO in toluene (5 ml) added and radioactivity monitored. The percentage inhibition by pyridine was calculated considering the binding in the experiment without pyridine as 100%.

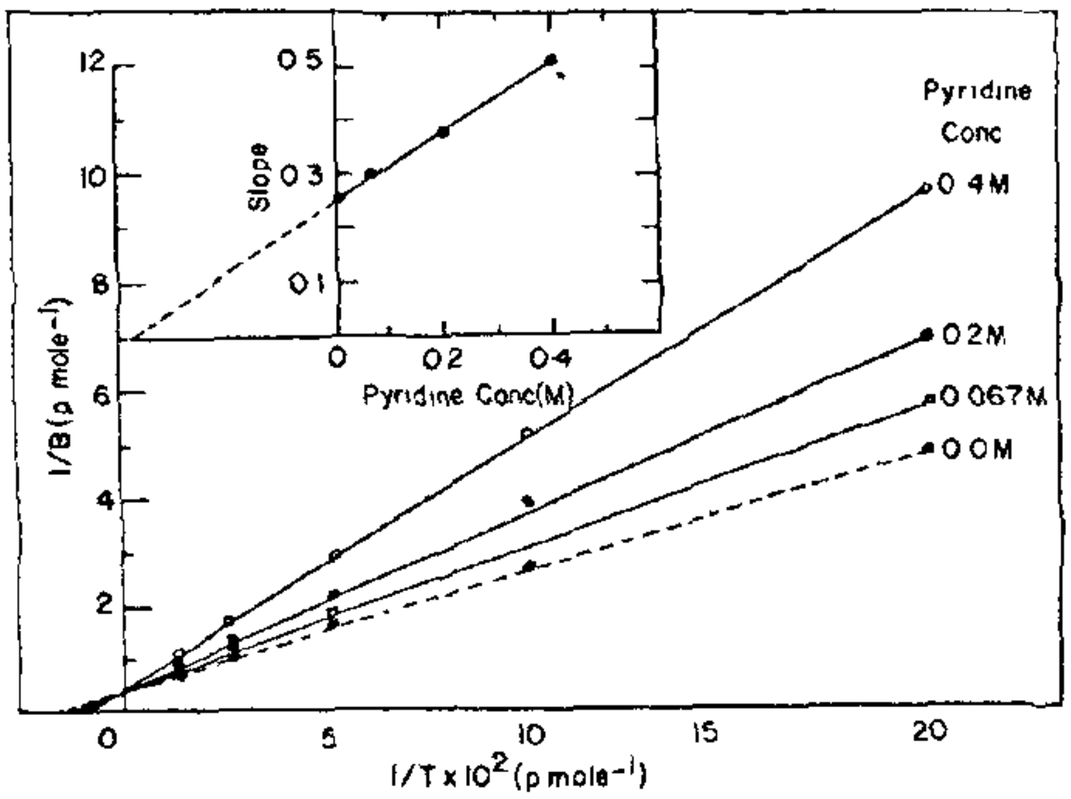

Figure 2. Kinetics of pyridine inhibition of ^{3}H-dpA binding to dpA antibodies. The reaction mixture (total volume 0.3 ml) contained purified dpA antibodies (12 μg), ^{3}H-dpA (various amounts) and TBS containing different concentrations of pyridine as indicated. The antibodies were added last. Incubation was at 37°C for 10 min. The nitrocellulose filter assay was as in figure 1. T is the concentration of the total ^{3}H-dpA used, and B is the concentration of the ^{3}H-dpA bound. The figure gives the data in double reciprocal plots. The insert gives the change in the value of slope with varying pyridine concentrations.

per molecule of hemocyanin as determined from difference spectra and protein content. Antibodies were raised in three rabbits, using LPH-dpA as immunogen and the dpA antibodies were purified on AH-Sepharose-dpA affinity column by dpA elution[9]. Binding of ^{3}H-dpA to the antibodies was assayed by the nitrocellulose filter method[8].

Figure 1 shows the percentage inhibition of ^{3}H-dpA binding to purified dpA antibodies at different concentrations of pyridine in TBS. Ten percent (1.24 M) pyridine brings about 95% inhibition of the binding. The inhibition was then studied at four pyridine concentrations using at each pyridine concentration the same amount of antibodies and varying inputs of ^{3}H-dpA. The results are given in figure 2 as double reciprocal plots. At different pyridine concentrations the intercept remains constant but the slope changes, showing that the inhibition is of competitive type.

The above findings that pyridine is a competitive inhibitor for ^{3}H-dpA binding to anti-dpA antibodies mean that some antigenic determinants of dpA have structural similarity to pyridine. The planar pyrimidine ring of adenine is likely to be this antigenic determinant.

2 May 1984

1. Lowe, C. P. and Dean, P. D. C., *Affinity Chromatography*, John Wiley and Sons, London, 1974.
2. Humayun, M. Z. and Jacob, T. M., *Biochim. Biophys. Acta*, 1974, **349**, 85.
3. Jayabaskaran, C., Sugumaran, M. and Jacob, T. M., *Curr. Sci.*, 1979, **48**, 666.
4. Reddy, M. V. and Jacob, T. M., *Indian J. Biochem. Biophys.*, 1983, **20**, 321.
5. Jayaraman, K. and Jacob, T. M., *Mol. Immunol.*, 1980, **17**, 769.
6. Reddy, M. V. and Jacob, T. M., *Indian J. Biochem. Biophys.*, 1983, **20**, 183.
7. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
8. Humayun, M. Z. and Jacob, T. M., *Biochim. Biophys. Acta*, 1973, **331**, 41.
9. Honnaiah, S., Ph.D. Thesis, Indian Institute of Science, Bangalore, 1981.

SURMASPORA, A NEW PTERIDOPHYTIC SPORE GENUS RECOVERED FROM THE TERTIARY SEDIMENTS OF MEGHALAYA AND ASSAM

H. P. SINGH and M. R. RAO
Birbal Sahni Institute of Palaeobotany, Lucknow 226 007, India.

DURING the course of palynological investigations of the Barail-Surma (Oligocene-Lower Miocene) sediments of Sonapur-Badarpur Road Section, Meghalaya and Assam, the present authors recovered quite a number of specimens of trilete miospores from the Upper Bhuban Formation (Dona Member) near 165.6 km stone on the Jowai-Badarpur Highway. This road section is located in the south east of Shillong. The miospores referred to the newly established spore genus *Surmaspora*, consists of a single species *viz. S. sinuosa*. In morphological characters *Surmaspora* has trilete rays surrounded by a thick labra having globular thickenings at the ray-ends. Exine is verrucose; verrucae are generally sparsely-spaced.

The slides and negatives are housed at the Birbal Sahni Institute of Palaeobotany, Museum, Lucknow.

Surmaspora gen. nov.

Type species—*Surmaspora sinuosa* gen. et sp. nov.

Generic diagnosis—Miospores triangular-subtriangular with broadly rounded apices. Trilete, γ-rays extend up to 3/4 of the spore radius surrounded by a thick labra having globular thickenings at the ray-ends. Exine verrucose, verrucae generally sparsely spaced.

Comparison—*Surmaspora* gen. nov. remotely compares with *Verrucosisporites* Ibrahim[1] and *Verrutriletes* (V. D. Hammen) Potonié[2] in having verrucose type of exine but can be easily distinguished from the former two genera by the presence of globular thickening at the ray-ends and ribbon-like labra. *Surmaspora* is closely comparable to *Dandotiaspora*[3] but differs from it in having verrucose exine and ribbon-like labra. *Garotriletes*[4] possesses foveo-reticulate ornamentation and hence it is not comparable. *Dictyophyllidites*[5] is different in possessing laevigate exine and distinct exinal thickening in close proximity to the trilete rays. *Biretisporites*[6] differs by having raised trilete mark which is almost covered by the upturned exine together with laevigate exine. *Sestrosporites*[7] possesses inter-radial thickenings along the trilete mark, thus it is not comparable. *Lycopodiumsporites*[8], *Foveosporites*[9], *Foveotriletes*[2]