17. Evans, C. T. and Ratledge, C., *Can. J. Microbiol.*, 1985c, **31**, 1000.
18. Guerritore, A. and Hanozet, G. M., *Experientia*, 1970, **26**, 28.
19. Evans, C. T. and Ratledge, C., *Lipid*, 1983a, **18**, 623.
20. Evans, C. T. and Ratledge, C., *J. Gen. Microbiol.*, 1984a, **130**, 1693.

Received 28 October 1991; accepted 10 January 1992

Antifertility and abortifacient activities of vicolides B and D

Muzaffer Alam, T. Susan, S. Joy and A. B. Kundu
Captain Srinivasa Murti Drug Research Institute for Ayurveda,
A. A. Government Hospital Campus, Arumbakkam,
Madras 600 106, India

Vicolides B and D isolated from *Vicoa indica* DC. showed antifertility activity in rats. The inhibition of implantation and abortifacient activities of vicolide D at 200 mg per kg body weight were found to be 52.43% and 71.43% respectively. The antiprogestational activity of vicolide D was evident from its ability to affect the deciduoma formation in the uterus of pseudopregnant rats. The mixture of vicolides B and D in the ratio of 1:1 at a dose of 100 mg per kg body weight showed 28.08% inhibition of implantation and 70% antifertility activities. It had 50% and 62.5% abortifacient activity from day 8 to day 14 and from day 14 to day 21 respectively.

Vicoa indica DC. of the family Compositae is used by the tribal women of Bihar as an antifertility agent. Of the four vicolides isolated from this plant, vicolides B and D had shown (Figure 1) antifertility and abortifacient activities in albino rats[1–4]. Vicolide D had 71% antiimplantation activity at a dose of 100 mg per kg body weight[4]. Further antifertility activity of vicolide D was assessed at a dose of 200 mg per kg body weight besides its antiprogestational activity at a dose of 100 mg per kg body weight.

Vicolide B causes resorption of implants[1,2] whereas vicolide D prevents implantation[4]. The amount of vicolides B and D present in the plant varies with the maturity of the plant. Our observations showed that vicolide B is present in good quantity before the flowering stage while vicolide D dominates during the peak flowering period. The plant at preflowering stage is fit for antifertility use according to field reports from observations in the tribal areas.

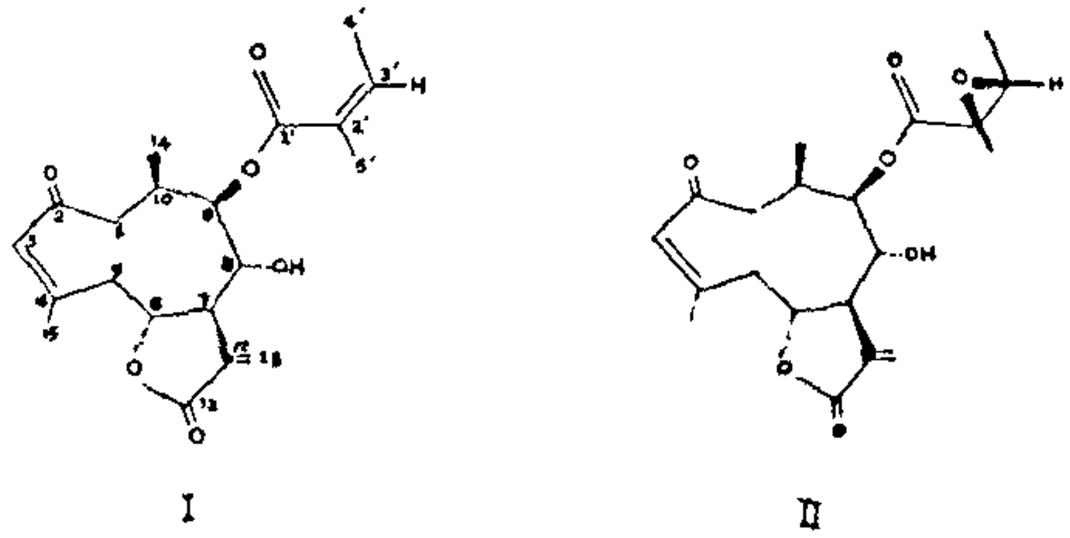
The fact that the whole herb is consumed by the tribals for contraception together with the difference in the degree and differential mode of action of the isolated vicolides prompted us to investigate the levels of combined activity of vicolides B and D.

The plant *Vicoa indica* DC. was collected from the neighbourhood of Madras city. Vicolides B and D were isolated according to the procedure detailed by Purushothaman *et al*[5,6].

Antiimplantation activity. Proven fertile female rats of Wistar strain weighing 150–200 g were screened for 2–3 oestrous cycles by examining the vaginal smears. The rats that showed normal cycles for two successive examinations were selected for the study. The method of Khanna and Chowdhuri[7] was followed with necessary modifications. The rats in prooestrous and oestrous stages were caged with fertile males in the ratio 3:1. The following day vaginal smears were examined and the appearance of sperm clusters in the smears was recorded as day 1 of pregnancy. Vicolide D was administered orally in suspension in 0.5% carboxy methyl cellulose at a dose level of 200 mg per kg body weight from day 1 to day 5. Control animals received vehicle only. On day 10 laparatomy was performed under light ether anaesthesia to examine uteri for implant number and size. Then the abdomen was closed and rats were allowed to recover and deliver after full term of pregnancy. Those rats that did not deliver were laparatomized on day 25 and uteri were examined for implantation sites. The number of implants present in vicolide-D-treated rats on day 10 was compared with the control group rats to determine the per cent of inhibition of implantation.

Abortifacient activity. The method of Khanna and Chowdhuri[5] was followed with necessary modifications as described earlier. Rats at day 1 of pregnancy were divided into two groups. The first group served as control and was fed vehicle only while the second group was administered vicolide D at 200 mg per kg body weight from day 14 to day 21 of pregnancy. Other experimental conditions were the same as detailed under antiimplantation study.

Antiprogestational activity. The antiprogestational activity of vicolide D was evaluated with reference to its ability to inhibit deciduoma formation in traumatized uterine horn of pseudopregnant rat. The experiment was carried out according to the method of Zarrow *et al*[8].



I II

Figure 1. Vicolides from *Vicoa indica* DC. I, vicolide B; II, vicolide D.

Virgin adult female rats of 100–150 g which showed regular cycles for at least four successive cycles were chosen. The animals were ovariectomized and one week later estradiol benzoate was injected subcutaneously at the dose of 0.1 μg per rat per day for three days. The ovariectomized estrogen-primed animals were divided into four groups of eight animals each. First group received vehicle only (0.5% carboxy methyl cellulose) in appropriate quantity and served as control. The second group received progesterone at the dose of 1 mg per rat per day subcutaneously. The third group received 1 mg progesterone subcutaneously and vicolide D orally at the dose level of 100 mg per kg body weight. The fourth group received vicolide D alone orally at 100 mg per kg body weight. This regimen of treatment was continued for nine days. In all the above groups all the animals underwent laparatomy on the fifth day of treatment. Histamine HCl solution (1 mg per rat) was administered into the lumen of one uterine horn of each rat to provide chemical stimulus for inducing deciduoma. On day 10 the animals were sacrificed, uteri weighed, fixed and sectioned at 6 μ and stained with haematoxylin and eosin for histopathological studies.

Vicolides B and D were mixed in the ratio 1:1 and the mixture was suspended in carboxymethyl cellulose. This mixture was screened for antiimplantation and abortifacient activities from day 8 to day 14 and day 14 to day 21 according to the procedure detailed above at a dose of 100 mg per kg body weight.

Vicolide D at a dose of 200 mg per kg body weight showed 52.43% inhibition of implants compared to 70.64% at 100 mg dose level. The abortifacient action from day 14 to day 21 was 71.4% which is higher than that reported for the dose of 100 mg per kg body weight which was 50%[4] (Table 1). The litters of vicolide D-treated animals were of normal weight.

Antiprogestational activity—Histopathology of uterus. Macroscopic. The control animals did not show difference in the size of traumatized and control horns. Massive decidual swellings were observed in the traumatized uterine horns of the progesterone-treated animals. There was significant increase ($P < 0.001$) in the weight of traumatized horns when compared to their respective contralateral control horns in progesterone group and progesterone + vicolide D-treated group (Table 2). The group treated with vicolide D + progesterone and the group treated with vicolide D alone showed significant decrease in the weight of traumatized horns compared to progesterone-treated animals.

Microscopic. The haematoxylin- and eosin-stained sections of uterine horn pair in control group and the control horns in all the groups receiving different treatment showed normal uterine histology with proliferative endometrial glands.

Under high power the test horns of progesterone-treated animals showed smaller, fewer and less prominent glands. The stromal cells were enlarged, but retained normal spindle shape with enlarged prominent nuclei. These cells—the decidual cells—were present in scattered bands or more compact groups as deciduoma. In some slides broad sheets of decidual cells in thickened layers of endometrium were seen (Figure 2).

In the groups treated with progesterone + vicolide D the formation of decidual cells was reduced. This retardation in the formation of decidual cells was indicative of the interference caused by vicolide D (Figure 3). In the test horns of vicolide-D-treated animals the cellularity was quite evident but the stroma was paler in that the cells were less crowded with a mild degree of intercellular oedema. The endometrial glands did not show any notable change.

It has thus been found that vicolide D has demonstrable antiprogesterone activity as evidenced from its effects on the morphology and histopathology of the uterus.

The present study has revealed that the antifertility activity of vicolide D is not dose-dependent. Further it has shown that it is antiprogestational in nature. The

Table 1. *Antiimplantation, abortifacient and antifertility activities of vicolide D (values are mean ± SD)*

Treatment	Dose (mg/kg body weight)	Period of treatment	Number of implants on day 10	Number of litters delivered on day 23	Inhibition of implantation (%)	Animals aborted* (%)	Animals with anti-fertility activity* (%)
Control (5)	–	–	7.80 ± 1.78	7.60 ± 1.81	Nil	Nil	Nil
Vicolide D (7)	50	day 1 to day 5	5.29 ± 4.15	2.56 ± 3.04[b]	32.17	Nil	71.4
Vicolide (7)	100	Day 1 to day 5	2.29 ± 3.91[a]	2.30 ± 3.90[b]	70.64	Nil	71.4
Vicolide (6)	100	Day 14 to day 21	6.0 ± 3.14	2.50 ± 4.28[b]**	Nil	50.0	50.0
Vicolide (7)	200	Day 1 to day 5	3.71 ± 3.86[b]	3.28 ± 3.41[b]	52.43	Nil	42.85
Vicolide (7)	200	Day 14 to day 21	6.0 ± 2.30	1.43 ± 2.40[c]**	Nil	71.43	71.43

*Animals with more than 50% resorption alone are taken as animals aborted 'animals with antifertility activity.
Values in paranthesis are the number of animals in each group.
Values are significant when $p < 0.05$, [a] < 0.02; [b] < 0.01; [c] < 0.001.
**Compared with the number of implants on day 10 of the same group.

Table 2. Antiprogestational activity of vicolide D at 100 mg per kg body weight (values are mean ± SD)

Group	Treatment	Control horn mg/100 g body weight	Traumatized horn mg/100 g body weight	Increase in weight (%)
I	Control (8)	33.47 ± 9.44	34.79 ± 10.36	3.90
II	Progesterone (8)	41.50 ± 3.67	284.18 ± 17.36[a]	584.72
III	Progesterone (8) + Vicolide D	30.74 ± 1.22	118.11 ± 5.32[a]	284.22
IV	Vicolide D (8)	31.78 ± 9.05	34.36 ± 8.46	8.11

Values are significant when $p < 0.05$; [a] < 0.001.
Values in parenthesis are the number of animals in each group.

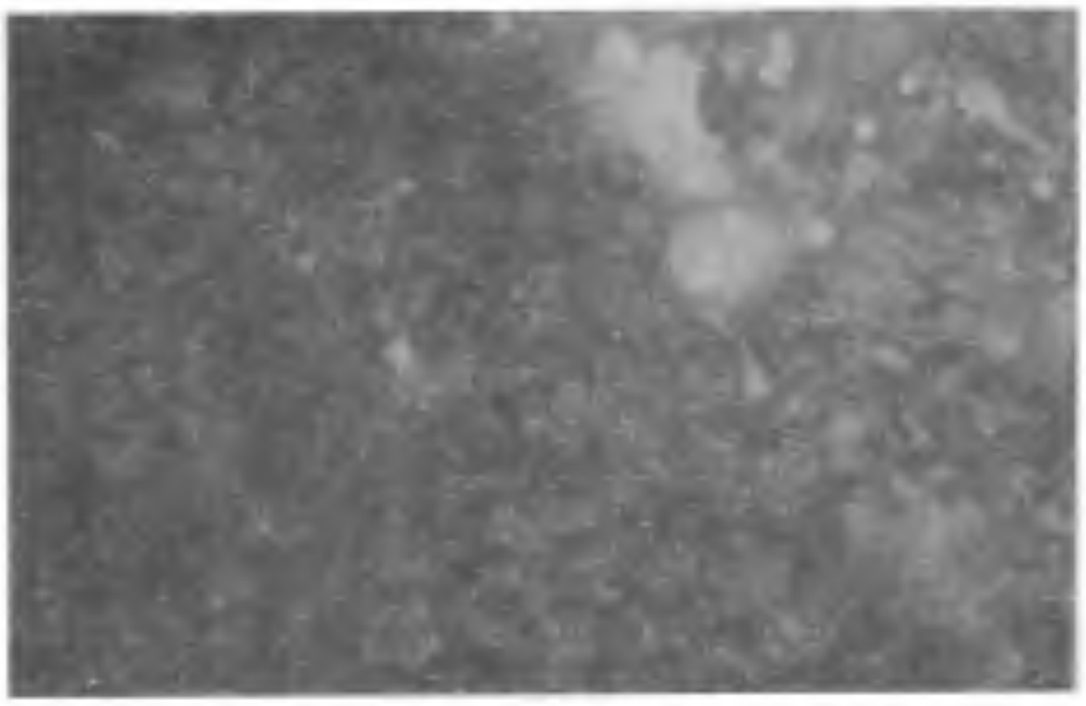


Figure 3. Traumatized uterine horn treated with progesterone and vicolide D.

Figure 2. Traumatized uterine horn treated with progesterone.

antioestrogenic nature of vicolide D is already reported[4]. The antiimplantation activity is probably due to its antioestrogenic nature and the abortifacient activity is due to its antiprogestational activity. Progesterone is necessary for the development of implants and its absence causes expulsion of implants.

Vicolides B and D in combination. The inhibition of implantation by the mixture was 28.08% while antifertility activity was 70% (Table 3). The abortifacient action from day 8 to day 14 was 50% while from day 14 to day 21 it was 62.5% (Table 3).

Vicolide B alone at the dose of 50 mg per kg body weight showed 87.5% and 100% antifertility and abortifacient activities respectively[1,2]. The antiimplantation activity at this dose was 25% for vicolide B and 28.6% for vicolide D[2,3]. The present observation of 28.08% inhibition of implantation by the mixture is in agreement with the earlier observations.

The antifertility activity of the mixture was 70%

Table 3. Antiimplantation and abortifacient activities of mixture of vicolides B and D (1 : 1) at 100 mg/kg body weight (values are mean ± SD)

Treatment	Period of treatment	No. of implants on day 10	No. of litters delivered on day 23	Inhibition of implantation (%)	Animals with antifertility activity* (%)	Animals aborted* (%)
Control (8)	–	7.37 ± 1.18	6.50 ± 1.77	Nil	Nil	Nil
Mixture of vicolides B and D (10)	Day 1 to day 5	5.30 ± 4.0	2.0 ± 3.23[a]	28.08	70	–
Mixture of vicolides B and D (10)	Day 8 to day 14	4.90 ± 4.01	3.10 ± 3.38[b]	–	50	50
Mixture of vicolides B and D (8)	Day 14 to day 21	7.50 ± 2.97	3.62 ± 2.87[a]**	–	62.5	62.5

Values in parenthesis are the number of animals in each group.
Values are significant when $p < 0.05$; [a] < 0.01; [b] < 0.02.
**Compared with the number of implants on day 10 of the same group.
*Animals with more than 50% resorption alone are taken as animals aborted, animals with antifertility activity.

when administered from day 1 to day 5. Further there was a significant reduction in the number of litters delivered on day 23 ($P < 0.01$) compared to the litters of the control group. This activity seems to be due to vicolide D because vicolide D alone had shown 71.4% antifertility activity at 50 mg per kg body weight[3]. The antifertility activity of vicolide B was 87.5% at this dose level[2].

The animals aborted on administration of vicolide B alone from day 8 to day 14 was 100%[1] but the mixture had shown 50% activity. The animals of this group showed significant reduction in the number of litters delivered on day 23 ($P < 0.02$) compared to the control group. The per cent animals aborted by the mixture on administration from day 14 to day 21 was in agreement with that of vicolide D at 100 mg per kg body weight[3].

This study suggests that the action of vicolide B is not fully exhibited when combined with vicolide D. This may be due to competition of vicolide D with vicolide B in the physiological system. Both vicolides may be substrates for the same enzyme because both were anioestrogenic in nature[2,3]. Chemically vicolides B and D are germacranolides. Vicolide D differs from vicolide B in having an epoxy group in the side chain. The antiprogestational activity exhibited by vicolide D is probably due to this group in the molecule. The inhibition of activity of vicolide B may also be due to this epoxy group in vicolide D. The diminished activity exhibited by vicolide B from day 8 to day 14 may be due to its inhibition by vicolide D. Vicolide D alone had shown negligible activity from day 8 to day 14 in rats at 100 mg per kg body weight[3].

We thus conclude that in a 1:1 vicolide B and D mixture the net antifertility activity will be due to vicolide D only.

1. Mohana, K., Susan, T. and Purushothaman, K. K., *Arogya—J. Health Sci.*, 1984, **10**, 42.
2. Susan, T., Alam, M and Purushothaman, K. K., *Indian Drugs*, 1985, **22**, 512.
3. Susan, T., Alam, M. and Purushothaman, K. K., *Indian Drugs*, 1986, **23**, 452.
4. Alam, M., Susan, T., Joy, S. and Rao, R. B., *Indian Drugs*, 1989, **26**, 337.
5. Purushothaman, K. K., Saradha, V., Cox, P. J., Akinniyi, J. A., Connolly, J. D., Rycroft, D. S. and Sim, G. A., *J. Chem. Research (S)*, 1981, 374.
6. Purushothaman, K. K. and Vasanth, S., *Indian J. Chem.*, 1986, *B25*, 417.
7. Khanna, U. and Chowdhuri, R. R., *Indian J. Med. Res.*, 1963, **56**, 1975.
8. Zarrow, M. X., Peters, L. E. and Calwell, A. Z., *Ann. N. Y. Acad. Sci.*, 1958, **71**, 532.

ACKNOWLEDGEMENTS. We thank Vaidya S. Usman Ali, CRI (Siddha), Madras, for useful discussions. We also thank the Director, Central Council for Research in Ayurveda and Siddha for facilities and financial support.

Received 31 August 1991; revised accepted 30 September 1991

Silanization of DNA bound baked glass permits enhanced polymerization by DNA polymerase

Radhashree Maitra and Ashoke Ranjan Thakur

Department of Biophysics, Molecular Biology and Genetics, University College of Science, 92 Acharyya Prafulla Chandra Road, Calcutta 700 009, India

DNA can bind to pure silicon dioxide through the formation of hydrogen bonds. This bonding occurs when the hydration shell of DNA is disrupted by chaotropic agents and such binding can be easily reversed with water or buffers of low ionic strength. Single-stranded, supercoiled and denatured DNA when bound to flint glass and baked at 60°C for 2 h permit synthesis reaction using Klenow. The reaction was enhanced when the glass was silanized prior to synthesis reaction.

DOUBLE-STRANDED helical structure of DNA has been analysed for its periodicity by binding it to calcium phosphate and then subjecting it to digestion by DNase I (ref. 1). This was the first report which showed that such solid-state-bound DNA maintains a periodicity which is greater than the one postulated for the B-DNA structure, viz. ten base pairs per turn of helix. The actual value turned out to be 10.4 and this was totally corroborated by the solution structure analysis of DNA. Hence mere binding of DNA to a solid state matrix is known not to distort the structure strongly. Also such structures were found to be amenable to nuclease digestion. Recently it has been shown that binding of photolabile 5′-nitroveratryl thymidine onto an aminated glass surface allowed solid state chemical synthesis of dinucleotides when treated with phospharamidite-activated 2′-deoxycytidine (ref. 2). We describe here activity of DNA polymerase (Klenow fragment) on glass-bound DNA.

The substrate chosen for analysis was single-stranded closed-circular DNA, double-stranded closed-circular duplex DNA (form I) and denatured closed-circular duplex DNA (form I_d). The objective being to investigate how far does binding to a two-dimensional surface influence the tertiary structure of the DNA to