8-H), 4.04 (s, broad, 5-H), 1.26 (d, superimposed, 14-H), 4.4–4.55 (m), 3.70 (s), 1.8–2.5 ppm (m) (OH, exchangeable). UV- and IR-spectra are similar to those of Cy_{14} (**1a**), whereas both, the molecular formula $C_{38}H_{58}O_{10}$ obtained by high resolution mass spectrometry and the subsequent elimination of 3 m/e = 116 fragments, indicate that Cy_6 is a trihexanoate of a dihydroxyingenol. As compared to Cy_{14}, the H-NMR-spectrum (chart 2) shows a downfield shift of 1-H and a new signal for 2 geminal protons at $\delta = 4.63$ ppm. The signal for 19-H_3 is missing and 14-H appears as a doublet at $\delta = 1.26$ ppm. From these findings it is obvious that the parent alcohol of Cy_6 is 13,19-dihydroxyingenol (**2**). The 3 acyl residues are structurally identical and were identified as 2,3-dimethyl-butyric acid by GLC (see above, factor Cy_{14}). By double resonance experiments (chart 2) they are shown to be associated with 3-O, 13-O and 19-O. Thus, Euphorbia factor Cy_6 is 3,13,19-tris-O-(2,3-dimethyl-butyryl)-13,19-dihydroxyingenol (**2a**).

An isomer of Cy_6 exhibiting similar mass spectroscopic fragmentation pattern and UV-spectrum is the compound Cy_2 (table, **2c**) with the following other data: IR (CH_2Cl_2): ν_{max}: 3580, 3550, 3460 (OH, 2960, 2930, 2870 (CH), 1720 cm^{-1} (C=O); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS} = 0.00$): 6.23 (s, broad, 1-H), 6.08 (d, broad, J = 4 Hz, 7-H), 4.45–4.95 (m, 19-H_2, 20-H_2, 3-H), 4.02 (dd, broad, 8-H), 3.64 (d, superimposed, 5-H), 4.23 (s), 3.74 (m), 3.03 ppm (d, broad) (OH, exchangeable). The complex group of signals around $\delta = 4.7$ ppm results from a diamagnetic shift of 3-H and a paramagnetic shift of 20-H_2 as compared to Cy_6 and allows to attribute to Cy_2 the structure of 13,19,20-tris-O-(2,3-dimethylbutyryl)-13,19-dihydroxyingenol (**2c**).

Cy_4 is another isomer of Cy_6 (chart 1, **2b**), but its IR-spectrum is similar to that of Cy_{13}. Also the 90 MHz ^{1}H-NMR-spectrum exhibits the characteristics of Cy_{13} and other 5-O-acylingenols[17]: ($CDCl_3$, $\delta_{TMS} = 0.00$): 6.21 (d, J = 6 Hz, 7-H), 6.03 (s, 1-H), 5.45 (s, 5-H), 4.71 (s, 19-H_2), 4.42 (dd, $J_{8,14} = 12$ Hz, $J_{7,8} = 6$ Hz, 8-H), 4.19 (s, broad, 3-H), 4.05 (s, 20-H_2). Thus, Cy_4 is 5,13,19-tris-O-(2,3-dimethylbutyryl)-13,19-dihydroxyingenol (**2b**).

The di-O-acylates of 13-hydroxyingenol are the most irritant and 13,19-dihydroxyingenol represents the most oxygenated ingenane type polyfunctional diterpene known so far. Some chemical reactions as well as the tumor promoting activity of these compounds, especially of the TPA-isomer Cy_{11}, will be reported elsewhere[19].

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Antimitotic effect of nimbidin – a first report

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Summary. The antimitotic activity of nimbidin, a drug from the plant *Melia azadirecta indica,* was assessed by its effect on the meristematic cells of onion root tips. The effect was almost similar to those of colchicine and vinca alkaloids. Recovery trials showed that the drug induces lethal damage in a considerable proportion of treated cells and may hence have applications in cancer chemotherapy.

The antimitotic effect of alkaloids such as colchicine, vincristine and vinblastine[1,2], has been exploited in cancer chemotherapy[3]. Screening of plant products for anticancer activity with a view to developing newer and more powerful anticancer drugs is in progress in several laboratories throughout the world[4]. We have also made considerable progress in this direction and data on the antimitotic effect of nimbidin, as obtained from the 'Allium test' of Levan[5], is summarized in this brief communication.

Nimbidin was isolated from the oil seeds of the plant *Melia azadirecta indica* by Siddiqui[6]. It is an amorphous cream-coloured water-insoluble granular powder of neutral character (yield is 1.1% w/v of oil). As nimbidin is insoluble in water, 1 g of nimbidin was dissolved in 10 ml of 10% ethyl alcohol in water and this was diluted further with distilled water to obtain 3 different concentrations (0.01%, 0.1% and 1%) of the drug. Rooted bulbs of onion *(Allium cepa)* were placed in the drug solutions for varying periods of time from 3–24 h, starting from 09.00 h. Soon after the treatment, the root tips were collected and fixed in Carnoy's fluid taken in separate tubes. These were squashed by the Feulgen technique for making cytological preparations. One batch of onion bulbs was treated for 24 h with the drug solutions and after washing, the bulbs were allowed to grow

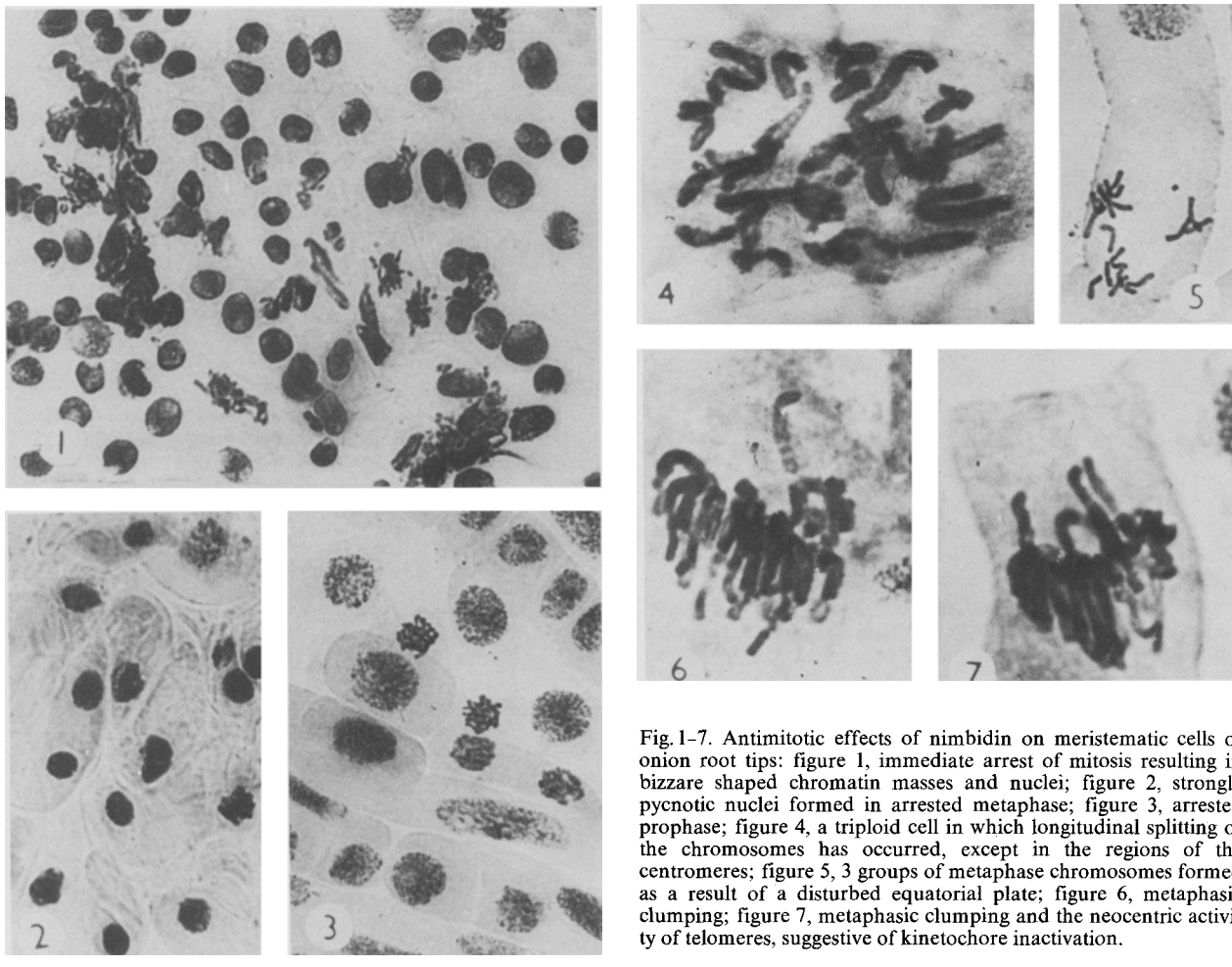

Fig. 1–7. Antimitotic effects of nimbidin on meristematic cells of onion root tips: figure 1, immediate arrest of mitosis resulting in bizzare shaped chromatin masses and nuclei; figure 2, strongly pycnotic nuclei formed in arrested metaphase; figure 3, arrested prophase; figure 4, a triploid cell in which longitudinal splitting of the chromosomes has occurred, except in the regions of the centromeres; figure 5, 3 groups of metaphase chromosomes formed as a result of a disturbed equatorial plate; figure 6, metaphasic clumping; figure 7, metaphasic clumping and the neocentric activity of telomeres, suggestive of kinetochore inactivation.

Table 1. Nimbidin-induced cytological abnormalities in onion root tip cells compared with controls

Concentration of nimbidin	Treatment time	Percentage of cells								
		Normal interphase	Interphase with nuclear lesions	Normal prophase	Arrested prophase	Normal metaphase	Arrested metaphase	Normal anaphase	Disturbed anaphase	Abnormal nuclei
0 (control)	09.00–12.00 h	62.25	Nil	23.50	Nil	8.75	Nil	2.30	1.28	1.92
	09.00–15.00 h	73.36	Nil	10.10	Nil	10.14	Nil	5.60	0.80	Nil
	15.00–15.00 h (24 h)	72.85	Nil	9.96	Nil	10.00	Nil	5.34	1.05	0.80
	15.00–15.00 h (48 h)	68.50	Nil	10.25	Nil	12.00	Nil	6.15	2.08	1.02
0.01%	09.00–12.00 h	48.05	10.25	12.50	8.80	4.78	5.25	3.40	3.17	3.80
	09.00–15.00 h	50.70	10.38	10.90	10.20	3.65	4.50	5.25	3.00	1.42
	15.00–15.00 h (24 h)	43.56	15.75	8.55	11.40	4.02	4.65	2.50	4.20	5.37
	15.00–15.00 h (24 h and recovery for 24 h)	71.75	12.90	3.20	2.50	2.00	2.95	0.70	1.00	3.00
0.1%	09.00–12.00 h	46.85	12.68	10.65	10.50	3.25	5.40	3.20	4.00	3.47
	09.00–15.00 h	44.00	14.25	8.50	11.00	3.60	4.75	4.55	5.25	4.10
	15.00–15.00 h (24 h)	42.35	16.00	7.85	11.50	2.95	5.55	3.50	5.00	5.30
	15.00–15.00 h (24 h and recovery for 24 h)	72.50	16.15	2.40	1.75	1.50	2.40	Nil	Nil	3.30
1%	09.00–12.00 h	45.05	15.00	3.60	12.50	2.25	5.75	2.75	4.20	8.90
	09.00–15.00 h	42.75	16.25	4.50	14.00	1.80	7.20	0.25	5.30	7.95
	15.00–15.00 h (24 h)	40.25	20.50	4.20	10.00	2.15	8.40	Nil	7.50	7.00
	15.00–15.00 h (24 h and recovery for 24 h)	62.75	20.00	1.75	2.00	0.85	3.00	0.40	Nil	9.25

in distilled water for 24 h and then the roots were fixed and squashed as above.

Mitotic arrest was the most salient feature of treated cells (figure 1). Deformed nuclei and nuclear lesions were also common in such preparations. Deeply pycnotic nuclei presumably formed by arrest in metaphase were also common (figure 2). Arrest in prophase and elongation of nuclei were also frequently encountered (figure 3). Early splitting of chromosomes was noticed and this in association with nondisjunction of daughter chromosomes could possibly result in polyploidy.

Figure 4 shows a triploid cell in which early splitting of chromosomes is very evident. Sister-strand exchanges were also observed. The spindle mechanism was found to be disturbed in the treated cells. The display of metaphase chromosomes in 3 different groups could also be noticed in some preparations (figure 5). Metaphase clumping (figure 6) and neocentric activity of telomeres (figure 7) were also encountered. In materials treated with the drug for 24 h and then allowed to grow in distilled water for another 24 h, there was a drastic decrease in the mitotic index. The cytological effects of different treatments with nimbidin are presented in table 1. (A minimum of 500 cells was observed for each timing and treatment.)

The Allium test was undertaken for rapid screening of drugs for antimitotic activity, since it is very convenient, inexpensive and provides hundreds of cells in mitosis in a single preparation. It is proposed to carry out further tests with sarcoma 180, adenocarcinoma 755 and leukemia 1210, according to the international scheme[7] for establishing the anticancer activity of this drug.

The results clearly indicate that nimbidin is a strong antimitotic agent. At higher concentrations, the drug is able to arrest cell division immediately upon treatment. The effect seems to be primarily in the kinetochore of chromosomes, as shown by the acute clumping of chromosomes and neocentric activity of telomeres. Disturbances in chromosome movement may be a secondary effect due to kinetochore inactivation. Such effects have been induced by UV-microbeam irradiation of kinetochores[8]. However, this does not interfere with the molecular and effective replications of chromosome strands. Thus this drug behaves similarly to colchicine and vinca alkaloids. From a comparison of these results with those obtained by us with adriamycin[9], it appears that nimbidin is a more powerful antimitotic agent than adriamycin, currently used in cancer chemotherapy.

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Vitellogenesis in the air breathing fish, *Channa punctatus* (Bloch)

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Summary. Two types of yolk develop in the oocyte of *Channa punctatus*. The carbohydrate yolk, which develops from the material present in the ooplasm, breaks up for the use of the growing oocyte before ovulation takes place. The proteid yolk, developing from the extraoocytic material, finally crams the fully mature oocyte, perhaps to participate in the process of embryogenesis.

Nath and Nangia[2], Chopra[3], Belsare[4], and Shahi et al.[5] have studied yolk formation in the oocyte of *Channa punctatus*, and Guraya[6] has studied it in *Channa maruleus*. An account of vitellogenesis in *Channa punctatus* is given here.

Material and methods. Specimens were collected from local ponds, and ovaries were processed[7] for the detection of proteins, carbohydrates, lipids and nucleic acids (table).

Results. 4 vitellogenic stages can be distinguished in developing oocytes: 1. in the young oocytes (160 µm in diameter), which appear as pre-vitellogenic ones in the preparations for histological studies and for detection of protein and lipid content, carbohydrate yolk develops (figure 1) as globules scattered randomly in the ooplasm; 2. when the oocyte measures 260 µm in diameter the carbohydrate yolk becomes vacuolated and starts to disintegrate, pouring its contents into the ooplasm (figure 2). This dissolution starts in the central zone and proceeds towards the peripheral ooplasm; 3. during the dissolution of the carbohydrate yolk, the accumulation of protein-containing precursor granules for the synthesis of another type of yolk, the proteid yolk, may be observed in the peripheral ooplasm (figure 3). These precursor granules eventually coalesce together, enlarge in size and change into proteid yolk, which gradually invades the whole ooplasm. Ultimately the carbohydrate yolk disappears and the mature oocyte is packed only with the proteid yolk.

Discussion. Yolks of varying structure and chemical nature develop in fish oocytes[8]. Thus, whereas 2 types of yolk, namely yolk globules and yolk granules, occur in *Liopsetta obscura*[9], *Carassius auratus*[10], and *Tilapia mossambica*[11], the mature oocyte of *Hypomesus japonicus*[12] contains 3 types of yolk: yolk vesicles, yolk globules and lipid globules. When the chemical nature is considered, it has been shown that two types of yolk - carbohydrate and proteid - develop in *Mystus tengara*[13], but Guraya[6] reports the elaboration of fatty yolk in addition to carbohydrate and proteid yolk in the oocyte of *Channa maruleus*. *Channa punctatus* develops 2 types of yolk; carbohydrate yolk developing de novo disintegrates in the growing oocytes and only proteid yolk, which is synthesized from the infiltrating protein-positive precursor granules, persists in the mature oocyte. It seems possible to visualize that the carbohydrate yolk is utilized by the growing oocyte before ovulation and the proteid yolk is used after fertilization during embryogenesis.