blocks, more developed in *S. buparius*. On the other hand, they share moderately asymmetrical karyotypes, high numbers of bivalents with interstitial chiasmata (8–10) and male X_1X_2Y sex-chromosomes. These show a morphology and a pattern of pairing which suggest that they are an homologous character for the 2 species and therefore must have arisen in a common recent ancestor. This point will be clarified by future studies of related species of this genus.

The fact that the X_1-chromosome shows a peculiar non-pairing arm without homologue in the Y-chromosome, and that this one and the X_2-chromosome show a higher degree of affinity (as judged by the mode of pairing), indicate that the X_1-chromosome was possibly the primitive X, the Y- and the X_2-chromosomes being of autosomal origin. This interpretation is thought to be more consistent than the one we proposed earlier about *S. buparius*[2].

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Tumorigenic potential of endoperoxide analogs

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Summary. The endoperoxide analogs known as U-46619 and U-44069 significantly enhanced the carcinoma formation and cellular atypicality initiated by a chemical carcinogen in mice. Studies of DNA radioactivity demonstrated that endoperoxides exerted their cocarcinogenic action by stimulating DNA synthesis. Thus, they play an important role in tumor cell proliferation.

Recent investigations suggest that prostaglandins play an important role in cancer development and the modulation of skin tumor promotion[2,3]. Some prostaglandins, such as PGD_2 and prostacyclin (PGI_2) also exert a cytoprotective activity and are powerful antimetastatic agents against B_{16} amelanotic melanoma colony formation in mouse lung[4,5].

The endoperoxide analogs known as U-46619 (15(s)-hydroxy-11α,9α-(epoxymethano) prosta-5Z, 13E-dienoic acid) and U-44069 (15(s)-hydroxy-9α, 11α-epoxymehtano) prosta-5Z, 13E-dienoic acid) are intermediary products in the synthesis of prostaglandins. They exert strong pharmacologic and physiologic effects on vascular and respiratory smooth muscle[6]. Thus, they are powerful vasoconstrictors, bronchoconstrictors and platelet aggregating agents[7].

As their role in carcinogenesis is unknown, this study was designed to investigate the effects of the stable endoperoxide analogs (U-46619 and U-44069) on tumor formation, DNA synthesis and cellular evolution of squamous cell carcinomas induced by 3-methylcholanthrene (MCA) in mice.

Materials and methods. The experiments were carried out on male Swiss mice weighing 25–30 g. They were divided into 6 groups of 20 mice as follows: 1. mice which received only the solvent and served as controls; 2. mice treated topically with 0.2 ml of a 0.3% acetone solution of MCA on a marked region of the shaved dorsal skin twice a week for 5 months; 3. mice treated topically with MCA as above and injected i.m. concomitantly with 5 µg of endoperoxide U-46619, twice a week; 4. mice treated with MCA as above and injected i.m. concomitantly with 5 µg of endoperoxide U-44069 twice a week. Groups 5 and 6 were treated only with U-46619 and U-44069 respectively, as above. The doses of U-46619 and U-44069 were similar to the dosage regimen used by other investigators in studying the bronchoconstrictor effect in dogs[8].

At the end of 5 months and 2 h before sacrifice under anesthesia with ether and nembutal, 6 mice from each experimental group received an i.m. injection of 7 µCi per g b.wt [^{3}H]-thymidine for the study of DNA synthesis. The period of 2 h prior to sacrifice was selected for the isotope studies because it was found in previous experiments that prostaglandins exert their maximum effect on cell structure and metabolism in that time[9].

DNA synthesis was comparatively studied in control epidermal and neoplastic cells from groups 2, 3 and 4, on at least 5–6 specimens, which were removed from experimental group, dissected, homogenized, using a Potter-Elvehjem homogenizer, and washed several times with 0.4 M per-

The incidence of carcinomas in mice following MCA and endoperoxide analog (U-46619 and U-44069) administration

Group	Treatment	Time (months)	No. of mice	Epithelial hyperplasia	Carcinomas	Percent of tumors
1	Controls + solvent	5	20	0	0	–
2	MCA + solvent	5	20	12	8	40
3	MCA + U-46619	5	20	0	20	100
4	MCA + U-44069	5	20	0	20	100
5	U-46619	5	20	10*	0	–
6	U-44069	5	20	9*	0	–

The data presented are based on counts of tumors visible to the naked eye, as well as on diagnosis made by light microscopy.
* Mild epidermal hyperplasia.

chloric acid, ethanol and ether[10]. Measurements were performed with a nuclear liquid scintillation system (Isotope-300; Searle Analytic) with an efficiency of 40%. DNA radioactivity was expressed as percent of controls and per µg of tumor cells or control epidermal cells. For light microscopic autoradiography the specimens were fixed in Bouin's solution, dehydrated and embedded in paraplast; sections 5 µm thick were stained with hematoxylin and eosin and covered with Kodak Nuclear Emulsion NTB_2 and exposed for 14 days after which they were developed in D_{19} Kodak developer, fixed and washed. Quantitative estimation of autoradiograms was performed by counting the labeled cells (under Zeiss microscope at ×400) from 2000 consecutive epithelial cells in the basal layers of control mice or in the proliferative compartments of tumors. The percentage of labeled cells was also recorded. For electron microscopy, specimens from tumors were fixed in 3% cacodylate glutaraldehyde, then postfixed in 1% phosphate buffered OsO_4, dehydrated and embedded in a mixture of epon:araldite (1:1); ultrathin sections were examined under HS-8 electron microscope.

Results and discussion. Multiple, sometimes coalescent and keratinized tumors in the center, occurred in almost 100% of both MCA plus U-46619 and MCA plus U-44069-treated mice as compared to only 40% in that of MCA-alone-treated mice. Tumors were increased numerically as well as in size in both endoperoxides- and MCA-treated mice (average tumor weight: 2100± 190 mg) as compared to that of MCA-treated mice only (730± 80 mg). No tumors were observed in mice treated with endoperoxide alone or in control mice (table). DNA synthesis expressed as percent of controls and per µg of tissue showed marked changes in the neoplastic cells following endoperoxide U46619 and U-44069 administration. Thus, DNA radioactivity was notably increased following MCA and endoperoxide analogs as compared to control mice or to that treated with endoperoxide only (fig. 1).

Light microscopic autoradiography revealed a significant increase (6-fold) or 49% of the heavily [^{3}H]-thymidine labeled nuclei of the neoplastic cells following MCA plus U-46619 or U-44069 administration, compared to that seen in controls (8%) or in MCA-treated mice alone (20%). Ultrastructurally, typical squamous carcinoma cells with large nuclei, nucleoli and also a large population of tonofilaments and polysomes can be seen in MCA-treated mice alone (fig. 2). Anaplastic cells of invasive type with numerous cell extensions, atypical nuclei, several dense granules (lipid droplets?) and poorly differentiated endoplasmic reticulum are predominately seen in mice treated with MCA and endoperoxides (fig. 3). These tumors resemble to atypical squamous cell carcinomas.

The findings presented here clearly demonstrate that the endoperoxide analogs U-46619 and U-44069 significantly enhanced the carcinoma formation, DNA synthesis as well as the cellular evolution towards more invasive and malignant type of tumor. Both endoperoxides U-46619 and U-44069 are not carcinogenic by themselves, since administration of endoperoxide alone induced only epidermal cell hyperplasia; thus they act in a manner similarly to most cocarcinogens, by enhancing the carcinogenic activity of MCA. It seems likely that endoperoxide analogs exert their

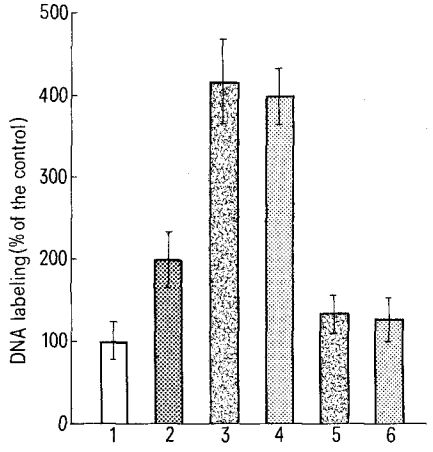

Figure 1. DNA radioactivity of control mice taken as 100 (1); MCA-treated mice (2); MCA + U-46619 (3); MCA + U-44069 (4); U-46619-treated mice (5); and U-44069-treated mice (6). The vertical bars at the top of each column represent the standard error of the mean (mean ± SE).

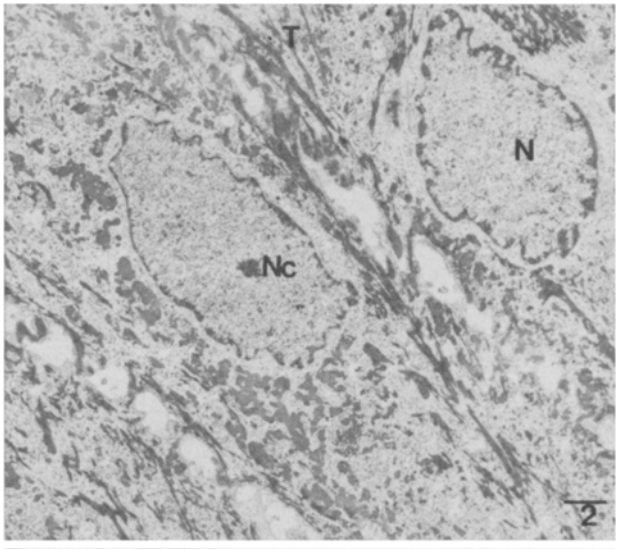

Figure 2. Electron micrograph showing characteristic squamous neoplastic cells with large nuclei (N), nucleoli (Nc) and several tonofilament (T) can be seen in mice treated with MCA alone. ×5400.

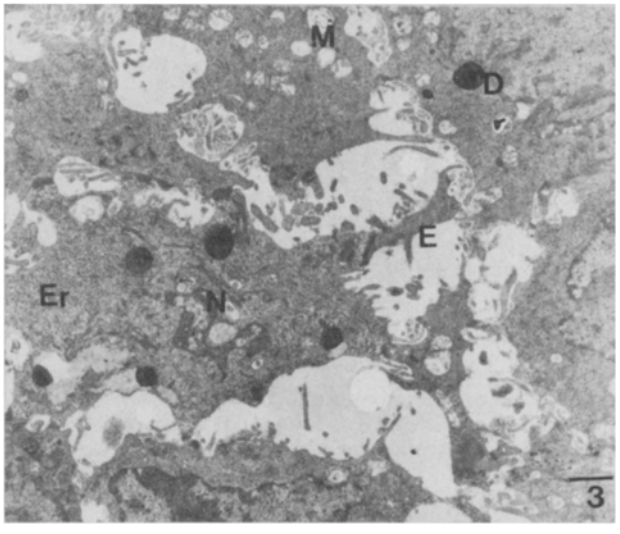

Figure 3. Electron micrograph showing anaplastic cell tumor composed of dense granules (D), poorly differentiated endoplasmic reticulum (Er), swollen mitochondria (M), and many cell extensions (E) can be seen in mice treated concomitantly with MCA + U-46619. ×5400.

tumorigenic potential by stimulating DNA synthesis. Both autoradiograms and DNA labeling revealed a significant increase in DNA synthesis (approximately 4-6 fold) in the nuclei of neoplastic cells following endoperoxide plus MCA administration compared to that of MCA alone, or control epidermal nuclei. It is also possible that endoperoxide analogs and thromboxanes accelerate the tumor cell growth by modulating the basal tumor cell cAMP levels[11]. Ultrastructurally, these are poorly differentiated and more invasive neoplastic cells.

The experiments described here conclusively implicated the endoperoxide analogs U-46619 and U-44069 in the control of tumor cell proliferation and function. Thus, research regarding the role of prostaglandins and their intermediary products, endoperoxide analogs, became an exciting field in oncology.

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The sperm attractant of *Hormosira banksii* (Phaeophyceae, Fucales), a seaweed common to Australia and New Zealand[1]

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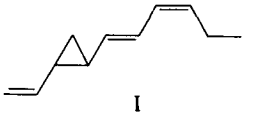
Summary. Hormosira banksii is a taxonomically isolated brown seaweed endemic to Australia and New Zealand. The sperm attractant of this species has been isolated and identified as trans-1-vinyl-2-(1*E*,3*Z*-hexadienyl)-cyclopropane (**I**) (hormosirene). *Hormosira* is the first organism in which a cyclopropane derivative has been found to act as a hormone in sexual reproduction. The implication of this finding in relation to phylogeny and phytogeography is discussed.

Chemical communication during sexual reproduction is a well-known and widespread phenomenon in lower plants[3]. Within the last 10 years marine brown algae have proved to be exceedingly well suited to the study of this problem, and the chemical structures of seven sex hormone factors have been identified recently in joint efforts by biologists and chemists[4,5]. Monocyclic molecular structures have been found in the genera *Ectocarpus*[6], *Cutleria*[7], *Dictyota*[8], *Desmarestia*[9], and *Syringoderma*[10]. Linar conjugated olefines with chain lengths of 8 and 11 carbon atoms have been identified in the respective genera *Fucus*[11] and *Ascophyllum*[4] which belong to the family Fucaceae within the order Fucales. Their straight-chain character and identical stereochemistry reflect a close taxonomic or phylogenetic relationship[8,12]. All organisms used for this previous work were marine brown algae, mainly from the North Atlantic.

The temperate coasts of Southern Australia have an extremely rich marine algal flora which differs significantly from that of the northern hemisphere. This region is the center of diversity of the most advanced group of brown algae, the order Fucales, and the region from which they are believed to have originated and spread. For this reason it seemed desirable to include species from this area in our studies. This paper reports the identification of the sex attractant in a second family of the order Fucales, the Hormosiraceae.

Hormosira banksii (Turner) Decaisne is endemic to Southern Australia and New Zealand. It is the dominant fucalean species in the lower eulittoral zone of many shores. It is dioecious, and in contrast to many other species in the order Fucales, *Hormosira* plants are fertile throughout the year in most localities. Reproduction is oogamous. Antheridia and oogonia exude on to the surface when the thalli are emersed at low tide. Upon contact with seawater the oogonial membranes dissolve and liberate 4 eggs per oogonium. Each antheridium releases 64 spermatozoids, which swarm around the eggs in large numbers. Gamete release and fertilization have been described in detail by Osborn[13], Levring[14], and Forbes and Hallam[15].

Materials and methods. Hormosira plants were collected from Sorrento and Point Lonsdale, Victoria (Australia), and stored at +4 °C until required. Individual plants were rinsed with cold seawater and allowed to dry at room temperature for a few minutes until the gametangia began to exude. Female thalli were then immersed in cold seawater and agitated to obtain a suspension of eggs. Batches of eggs amounting to about 5 ml volume were added to 2 l of seawater in an extraction flask. Volatile compounds were extracted by the closed-loop stripping technique[16], and adsorbed on a filter consisting of 2 mg activated carbon. After extraction periods of 24 h the filters were eluted with 30 µl of dichloromethane. These extracts were then subjected to further analysis by glass-capillary gas chromatography and mass spectrometry.



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