the anomeric protons (H'−1: J = 7.8 Hz for (**3**) and J = 3.9 Hz for (**4**)). 17β-stereochemistry for both (**3**) and (**4**) was deduced on the basis of the coupling patterns (triplet-like) of 17α-hydrogen signals, since it was reported that 17α-hydrogen resonance of 17β-OH derivatives is observed as nearly a triplet, but 17β-hydrogen resonance of 17α-OH derivatives as a doublet[9]. Thus, the structures of the metabolites **A** and **B** were established as those shown in the formulae (**1**) and (**2**), respectively. Enzymatic hydrolysis of the ^{14}C-labeled[10] (**1**) and (**2**) supported the assigned structures. Incubation of (**1**) with a β-glucosidase preparation from sweet almonds (Boehringer Mannheim) in 10 mM acetate buffer (pH 4.8) at 25 °C for 4 h, released 17β-estradiol while incubation of (**2**) with an α-glucosidase preparation from yeast (Boehringer Mannheim, grade I) under the same condition afforded 17β-estradiol as well, although the reaction was incomplete[10].

The present work provides the first rigorous structure characterization of steroid glucosides isolated from arthropods, although the formation of steroid glucosides in arthropods has been previously described, but without identification of the structures[11]. The two glucosides (**1**) and (**2**) are hitherto unknown metabolites of 17β-estradiol[12]. In insects the transfer of glucose from UDP-glucose to phenolic groups has been reported[13]. Glycoside synthesizing activity toward steroid hormones is known in vertebrates[14], plants[15], and microorganism[16]. The formation of the β-glucoside and α-glucoside together suggests the occurrence of both β- and α-glucosyltransferases in silkworm ovaries. It will be of great interest to see whether the glucosides exist in the ovaries of the insect.

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The effect of the phytoalexins, lubimin, (-)-maackiain, pinosylvin, and the related compounds dehydroloroglossol and hordatine M on human lymphoblastoid cell lines[1]

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Summary. We have tested the effect of the phytoalexins lubimin, (-)-maackiain and pinosylvin and the related compounds dehydroloroglossol and hordatine M on the growth of the human lymphoblastoid cell lines Molt and Raji. (−)-maackiain, pinosylvin and dehydroloroglossol showed significant growth inhibitory action on the cells. Suppression of [^{3}H] thymidine and [^{3}H] leucine uptake was tested and noted in pinosylvin and dehydroloroglossol. The phytoalexins and related compounds are widespread in plants and provide a potential source of antineoplastic substances.
Key words. Phytoalexins; growth inhibitory, transformed cells.

The generic term phytoalexin has been developed to describe substances newly synthesized by infected plants which possess antifungal activity against a wide range of fungi[2,3] although now this view has been somewhat modified and indeed at present there is no universally accepted definition[4]. Their production by plants is stimulated not only by the fungi themselves but also by substances from fungal culture filtrates or from extracts of fungal tissues[5,6]. These substances are called 'inducers' or 'elicitors'. The phytoalexins themselves consist of a wide variety of low molecular weight compounds with a preponderance at present of isoflavanoid and isoprenoid compounds. Their inhibitory action on fungal growth is well documented[2,7] but their effects on mammalian systems have been investigated only cursorily[8]. Studies have been published suggesting a possible antineoplastic role of the sesquiterpenoid phytoalexin capsidiol and the dihydrophenanthrene phytoalexin orchinol[9], and of the preinfectionally occurring, antimicrobial sesquiterpene parthenolide[10] as well as the terpenoid dihydrophenanthrene juncusol[11]. The last of these compounds may be a phytoalexin but this has not yet been established. This report documents the effect of other phytoalexins, viz. lubimin, (−)-maackiain, and pinosylyin, on transformed human cell lines and their normal counterparts. The synthetic compound dehydroloroglossol, closely related to the orchid phytoalexin loroglossol, and the preinfectional antifungal factor hordatine M were also tested.

Materials and methods. Test substances. Lubimin, from potatoes inoculated with *Alternaria solani,* and hordatine M, from uninfected barley seedlings, were available from other studies[12,13]. (−)-Maackiain was prepared by known procedures[14,15] from trifolirhizin tetraacetate which was a generous gift from Prof. S.

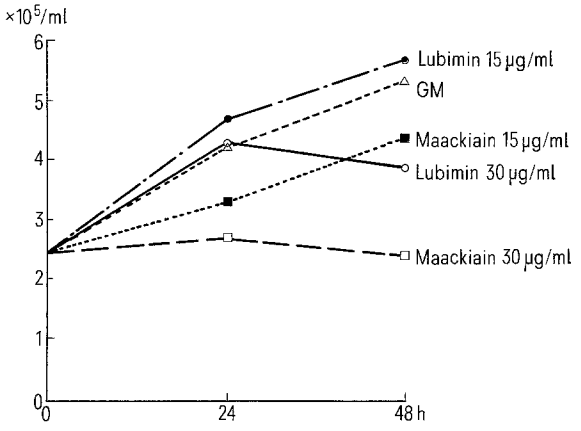

Figure 1. The effect of the phytoalexins lubimin and (−)-maackiain on the viability and growth of Molt cells over 48 h. GM represents cells grown in growth media alone.

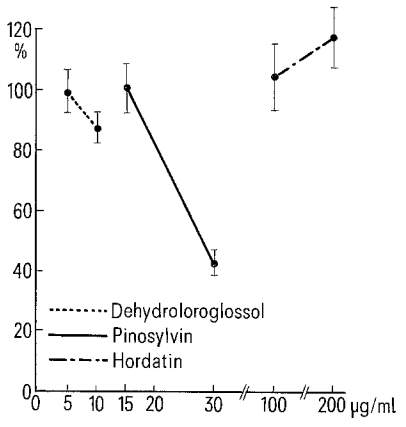

Figure 3. The effect of hordatine M, dehydroloroglossol and pinosylvin on the [^{3}H] thymidine uptake of Raji cells. The bars represent two standard deviations. The counts per minute for the control cells in growth media alone was 12,150.

Ito. Maackiain and trifolirhizin occur in various *Leguminosae*, the former frequently as a phytoalexin. Pinosylvin was synthesized by a published method[16] but occurs naturally in pine, as a phytoalexin, and in eucalyptus. Dehydroloroglossol, which has not yet been reported as a natural product, was prepared as described earlier[17], it is a close analogue of many known plant phenathrenes. Hordatine M readily dissolved in water. The other phytoalexins had to be dissolved in dimethylsulfoxide (DMSO) and were further diluted in RPMI 1640 (Grand Island Biological Co., N.Y.). The highest concentrations initially tested of lubimin, maackiain, pinosylvin and dehydroloroglossol were 30 µg/ml, 30 µg/ml, 30 µg/ml, and 10 µg/ml respectively as these were the concentrations at which solutions could be consistently maintained in 1% DMSO. This concentration of DMSO had no effect on the growth or viability of the cell lines and controls of cells with 1% DMSO were used in all tests. Hordatine M was tested up to 200 µg/ml. Raji and Molt cells were cultured in growth medium (GM, RPMI 1640 with 10% fetal calf serum) at an initial concentration of 2.5×10^5 cells per ml with and without varying concentrations of the compounds. Cell counts were carried out at 0, 24, 48 h. Viability was assessed with the trypan blue uptake. Cultures were carried out three times with all compounds. The significance of the results were tested by the Student t-test. For assessment of DNA synthesis hordatine M, pinosylvin and dehydroloroglossol were tested against Raji cells. Two $\times 10^5$ cells were cultured in triplicate in wells in Linbro plates with GM with and without various concentrations of the phytoalexins. After 18-h culture [^{3}H] thymidine was added for 4 h, and thymidine uptake was expressed as counts per minute (cpm). Protein synthesis was assessed in a similar way by the uptake of [^{3}H] leucine in cells grown in leucine free growth medium and also expressed in cpm. To compare their effect on normal as opposed to neoplastic cells their effect on the viability of normal lymphocytes was also tested.

Results. Apart from hordatine M all the compounds tested suggested some growth inhibitory action (figs 1, 2). This was least with lubimin which showed no inhibitory effect at 15 µg/ml and even at 30 µg/ml no statistically significant difference was evident (fig. 1). Statistically significant differences were found between the GM control and maackiain 30 µg/ml ($p = 0.025$), dehydroloroglossol 10 µg/ml ($p = 0.025$), pinosylvin 15 µg/ml ($p = 0.25$) and pinosylvin 30 µg/ml ($p = 0.005$). Pinosylvin appeared to be the substance with greatest cytotoxicity but it must be noted that this was at a concentration of 30 µg/ml while dehydroloroglossol could only be tested at 10 µg/ml. The effect on [^{3}H] thymidine uptake was assessed with hordatine M, dehydroloroglossol and pinosylvin. Again hordatine M even at the concentration of 200 µg/ml had no inhibitory effect (fig. 3). Pinosylvin at 30 µg/ml depressed the [^{3}H] thymidine uptake by more than 50% (fig. 3). Protein synthesis as assessed by [^{3}H] leucine uptake was inhibited to a greater degree than [^{3}H] thymidine uptake (fig. 4). Only dehydroloroglossol and pinosylvin were

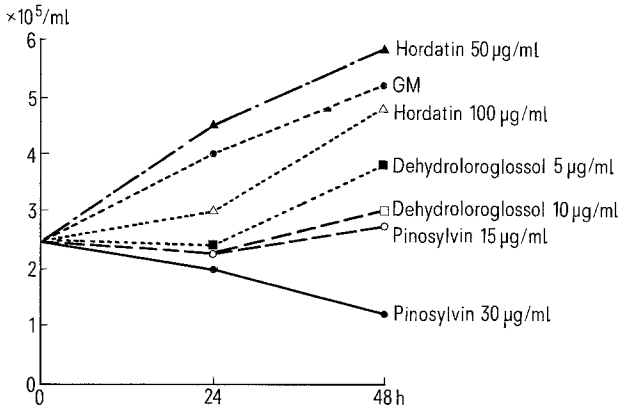

Figure 2. The effect of hordatine M, dehydroloroglossal and pinosylvin on the viability and growth of Raji cells over 48 h. GM represents cells grown in growth media alone.

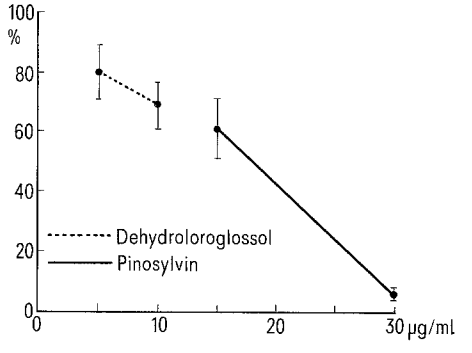

Figure 4. The effect of dehydroloroglossol and pinosylvin on the [^{3}H] leucine uptake of Raji cells. The bars represent two standard deviations. The counts per minute for the control cells in leucine free growth media alone was 33,362.

tested with the [^{3}H] leucine uptake. Pinosylvin at 30 µg/ml almost completely suppressed the [^{3}H] leucine uptake (fig. 4). The effect of all these compounds at the same concentrations on normal human peripheral blood lymphocytes was tested and at 72 h showed no effect on viability.

Discussion. Many of the antineoplastic agents now in use are derived from plants. Continual search for new biosynthetic substances with possible antineoplastic action must be maintained if progress in therapy of malignancies is to continue. Of the compounds tested all, except hordatine M and lubimin showed significant growth inhibitory action at at least the higher concentration tested. It should be noted, however, that dehydroloroglossol could only be tested at a concentration of 10 µg/ml while the others were tested at 30 µg/ml. With pinosylvin a cytotoxic action was noted at a concentration of 30 µg/ml. Pinosylvin was also shown to have a marked inhibitory action on protein synthesis as assessed by the [H^3] leucine uptake (fig. 4) and a similar effect though to a lesser degree on the [^{3}H] thymidine uptake (fig. 3). Dehydroloroglossol at 10 µg/ml had a slight but significant inhibition of both [H^3] leucine and [H^3] thymidine uptake (figs 3, 4). It is interesting to note that hordatine M was the only constituent tested at high concentrations which was not growth inhibitory or cytotoxic. Hordatine M is the only plant constituent tested which was not stress induced. No toxicity was noted on the normal mononuclear cells tested.

The phytoalexins and related substances open up a vast new field of biosynthetic substances that should be explored in search of new antineoplastic agents.

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Mitochondrial activity: A possible determinant of anoxic injury in renal medulla

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Summary. In brain[1], heart[2] and kidney[3], cell work in the absence of oxygen has been thought to precipitate anoxic damage by increasing the rate of depletion of cellular energy stores. In the medullary thick ascending limb of isolated perfused rat kidneys, however, reduction of ATP synthesis by a variety of mitochondrial or metabolic inhibitors caused ATP depletion comparable to that produced by oxygen deprivation but did not reproduce the lesions of anoxia. In these cells, unrestrained mitochondrial activity may be an important source of anoxic injury.

Key words. Anoxic injury; mitochondrial respiration; renal medulla; acute renal failure; renal metabolism.

Cell work under hypoxia has recently been shown to accelerate anoxic damage in isolated neurons[1], in myocytes[2], and in the medullary thick ascending limb of Henle's loop (mTAL) of isolated perfused rat kidneys[3]. Increased energy expenditure under these circumstances might hasten the depletion of cellular energy stores, generally considered an important element in anoxic cell injury[4]. The present study suggests that apart from its role in depleting cellular ATP, mitochondrial activity per se may contribute to the generation of cell injury during anoxia.

Severe hypoxic injury develops rapidly and consistently in isolated rat kidneys perfused with bovine albumin in Krebs-Ringer-Henseleit medium equilibrated with oxygen[5]. The lesion is located in the mTAL which, because of its strategic location, may play an important role in the pathogenesis of acute renal failure[6]. The selective vulnerability of the mTAL to anoxia results from its high transport activity combined with a meager oxygen supply[5]. The damage can be prevented by reducing reabsorptive transport and oxygen demand (adding ouabain or furosemide)[7] or can be intensified by increasing active electrolyte transport (adding a polyene antibiotic)[3]. Cell death under these circumstances thus appears to be mediated by increased energy demand for transport.

To evaluate the role of cellular energy depletion in the genesis of this injury, we examined the morphology of the mTAL in kidneys in which ATP production was reduced by inclusion in the perfusate of various mitochondrial or metabolic inhibitors, as follows. 1) Rotenone, antimycin (inhibitors of electron transfer at different proximal sites of the mitochondrial respiratory chain and known to deplete cellular ATP)[8] or oligomycin (inhibitor of oxidative phosphorylation). 2) Monofluoroacetate, malonate (blockers of the citric acid cycle)[9] or 2-deoxyglucose (which inhibits ATP production by interfering with the metabolism of glucose)[10]. 3) A combination of rotenone or antimycin with 2-deoxyglucose (to suppress both aerobic and anaerobic production of ATP).

For comparison, oxygen deprivation was achieved either by equilibrating the perfusion medium with nitrogen or by adding potassium cyanide (which prevents the binding of oxygen to the mitochondrial cytochrome a; a_3). Under these conditions, extensive injury occurs in the mTAL[11].

The effects of these probes were monitored by measuring tissue ATP content and oxygen consumption by the isolated perfused kidney. As expected, the various inhibitors effectively depleted the ATP content of the renal medulla (table) and significantly reduced oxygen uptake by the isolated kidney (fig. 1).

After 90 min of control perfusion, an anoxic lesion[5] was obser-