tal studies of the afferent responses and physiological mechanisms of the semicircular canals[12-15] and inertial receptors[16-19]. The presence and distribution of magnetic otoconia might indicate an additional functional role based on either the increased density of magnetic otoconia, or a magnetic sensitivity of the sensory cells. Whether or not the magnetic otoconia are functionally useful to the animal is presently unclear and requires further investigation.

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Highly irritant ingenane type diterpene esters from *Euphorbia cyparissias* L.

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Summary. From the roots of *Euphorbia cyparissias* L. a highly irritant diterpene ester fraction was isolated and further resolved into its constituents. Together with some less active and some inactive isomers, the pure Euphorbia factors were characterized as new diesters of 13-hydroxyingenol and as triesters of the new 13,19-dihydroxyingenol. The Euphorbia factors Cy_6, Cy_{11} and Cy_{14} are the strongest irritants of the ingenane ester type hitherto known, Cy_{11} being at least as active as the standard diterpene ester type irritant 12-O-tetradecanoylphorbol-13-acetate (TPA).

The cypress spurge (*Euphorbia cyparissias* L.), a small herb with narrow leaves, is indigenous to Europe, except for the extreme Northern and North-Eastern parts. It has been introduced into North America[3] and New Zealand[4]. Since the 4th century BC the use of the latex or other parts of the plant has been known in traditional medicine[5], especially against warts and other cancerous conditions[6]. Even today an ethanolic extract of the plant is recommended in homeopathy as a purgative and for treatment of psoriasis, diarrhoea, inflammations and rheumatic diseases[7].

In our research program on the distribution of co-carcinogens (i.e. tumor promoters[8]) in the plant kingdom[9], at the beginning the large succulent Euphorbiaceae of the tropical regions of the world were studied for obvious reasons (e.g. Opferkuch and Hecker[18]). With the experience accumulated we started to investigate the rather small herbaceous, European *Euphorbia* species, such as *Euphorbia lathyris* L.[15] and *Euphorbia cyparissias* L. Investigation of the latter species was stimulated by reports indicating that it contains a toxic and irritant principle, which causes acute poisoning of cattle if the plant occurs in hay[3,10]. In acute or chronic intoxication of this kind a transfer of the irritant principle into milk may take place[3,10]. Also, from the closely related[3] herbaceous *Euphorbia esula* L. the isolation of the antileukemic 3,20-di-O-benzoylingenol[11] and aliphatic O-acylated esters of ingenol[12] has been reported. Evidence has been presented[13] that the biologically active diterpene esters contained in *Croton flavens* L. may be environmental risk factors for esophageal cancer, in connection with its local use on Curacao for a popular 'bush tea'. Hence, considering the background of its reported utilization, it appeared well worth while to describe more accurately the diterpene constituents of *E. cyparissias* L.

Diterpene ester fraction from roots. The extract of fresh roots of *Euphorbia cyparissias* L., obtained by exhaustive extraction with methanol at room temperature, is an irritant as measured by the irritant dose 50 (ID_{50}) on the mouse ear[14]. The methanolic extract is distributed between dichloromethane and water. From the organic phase, by 2 subsequent O'Keeffe distributions[14] in different solvent systems and by subsequent column chromatography of the active portion on silica gel, a diterpene ester fraction is prepared. From this fraction containing all the irritant activity pure diterpene esters can be isolated by multistage Craig distribution[14] followed by TLC of the active fractions. All pure compounds isolated are colourless resins, resisting all attempts at crystallization, so far. Altogether, according to their partition coefficients, they comprise 2 groups of polyfunctional diterpenes.

Di-O-acylates of the polyfunctional diterpene parent 13-hydroxyingenol. (**1**). The main component of the 1st group of polyfunctional diterpenes is the irritant Euphorbia factor Cy_{14} (table, **1a**): MS: 644 (M^+), 626 (M^+-18), 510 (M^+-134), 426 (M^+-218), 310 (base peak); IR (CH_2Cl_2): ν_{max}: 3580, 3550, 3510, 3420 (OH), 2925, 2850 (CH), 1722 cm^{-1} (C=O); UV (CH_3OH): $\lambda(\varepsilon)$: 193 nm (17180); $\lambda_{max}(\varepsilon)$: 291 nm (220); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS}=0.00$):

6.05 (s, broad, 7-H, 1-H), 5.47 (s, 3-H), 4.18 (s, broad, 20-H_2), 4.1 (mc, 8-H), 4.08 (s, 5-H), 1.80 (s, 19-H_3) 4.45, 3.6, 2.0–2.9 ppm (OH, exchangeable). The UV-absorption and the ^{1}H-NMR-spectrum are very similar to those of 3-O-acylingenols[15], but the molecular formula $C_{38}H_{60}O_8$ (high resolution mass spectrometry) indicates the presence of an additional hydroxyl group and a 2nd acyl residue. According to fragmentation, the 2 acyl residues are a dodecanoic and a hexanoic acid (200 and 116 mass units). Following reductive cleavage of Euphorbia factor *Cy*$_{14}$ with $LiAlH_4$, the alcohols corresponding to the acid moieties were investigated by GLC. On Triton X-100 and GESE 30 the C_6-alcohol is identified as 2.3-dimethylbutanol by comparison with all isomeric C_6-alcohols. The retention time of the C_{12}-alcohol (on GESE 30) is slightly shorter than for dodecanol. This suggests that the C_{12}-acid of Euphorbiafactor *Cy*$_{14}$ may be methyl branched. Because no new downfield ^{1}H-NMR signal for a geminal proton is observed in *Cy*$_{14}$ as compared to ingenol-3-acylates, the new hydroxyl group must be tertiary and is esterified with the 2nd acyl residue.

A minor component of the diterpene ester fraction is the non-irritant compound *Cy*$_{12}$ (table, **1c**), which has the same

Irradiated at	δ [ppm]	Observed at	δ [ppm]	Change in multiplicity	Cancelled coupling
7-H	6.07	8-H	4.01	dd → d (J = 13 Hz)	4 Hz
		5-H	3.67	Sharpening	-
8-H	4.01	7-H	6.07	d → s (broad)	4 Hz
14-H	1.30	8-H	4.01	dd → d (J = 4 Hz)	13 Hz

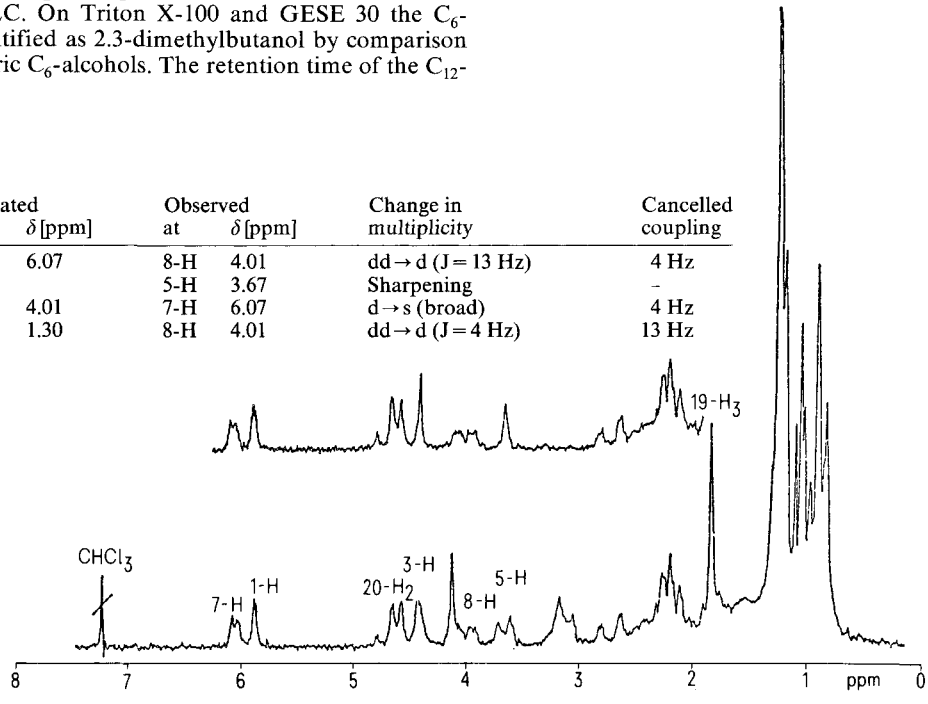


Chart 1. 90 MHz ^{1}H-NMR.-spectrum of compound *Cy*$_{12}$ (**1c**), in $CDCl_3$. At the top: in $CDCl_3 + D_2O$, with double resonance experiments.

Irradiated at	δ [ppm]	Observed at	δ [ppm]	Change in multiplicity	Cancelled coupling
1-H	6.32	19-H_2	4.63	Sharpening	-
19-H_2	4.63	1-H	6.32	Sharpening	-
7-H	6.01	20-H_2, 8-H, 5-H	4.1	Sharpening	-
20-H_2, 8-H, 5-H	4.1	7-H	6.01	d → s	4 Hz
		14-H	1.26	d → s	(Superimposed)

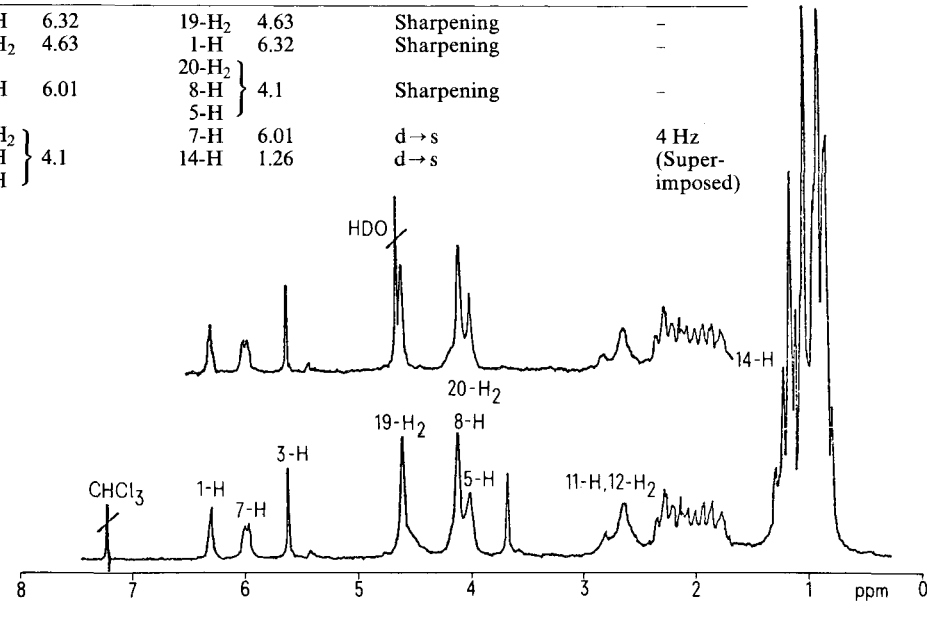


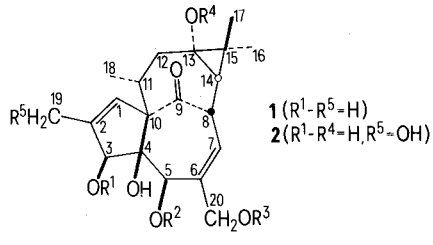
Chart 2. 90 MHz ^{1}H-NMR.-spectrum of Euphorbia factor *Cy*$_6$ (**2a**), in $CDCl_3$. At the top: in $CDCl_3 + D_2O$, with double resonance experiments.

molecular formula as Cy_{14}, and a similar mass spectroscopic fragmentation pattern and UV-absorption. Other spectral data are different: IR (CH_2Cl_2): ν_{max}: 3590, 3540, 3470 (OH), 2925, 2855 (CH), 1720 cm^{-1} (C=O); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS}=0.00$; see also chart 1): 6.07 (d, broad, J=4 Hz, 7-H), 5.88 (d, J=1.5 Hz, 1-H), 4,64±0.05 (AB, $J_{AB}=12$ Hz, 20-H_2), 4.43 (s, broad, 3-H), 4.01 (dd, broad, 8-H), 3.67 (d, broad, J=10 Hz, 5-H), 1.85 (d, J=1.5 Hz, 19-H_3), 1.30 (d, superimposed, 14-H), 4.12 (s), 3.2 (s, broad), 3.1 ppm (d, broad) (OH, exchangeable). The ^{1}H-NMR-spectrum is very similar to those of 20-O-acylingenols[15]. According to these data, the 3-O-acyl residue of Cy_{14} is in Cy_{12} translocated to 20-O. The new tertiary hydroxyl group of Cy_{12} and Cy_{14} must be attached at C-13, because the other possible atoms C-8, C-11 and C-14 are carrying a proton. This is obvious from the chemical shift of 14-H and can be demonstrated by nuclear magnetic double resonance experiments (chart 1). Although the structure of Cy_{12} is identified without doubt as the 13,20-di-O-acylingenol **1c**, its ^{1}H-NMR-spectrum (chart 1), especially the signal of 5-H, differs for unknown reasons from the spectrum of an isomeric compound isolated from *Euphorbia kansui* Liou[16]. Another isomer of Cy_{14} and Cy_{12} is Euphorbia factor Cy_{13} (table, **1b**) which, in different systems, shows R_f-values nearly identical with those of Cy_{14}. Mass spectroscopic fragmentation pattern and UV-spectrum are very similar to those of Cy_{14} (**1a**) and Cy_{12} (**1c**). Other data are as follows: IR(CH_2Cl_2): ν_{max}: 3590, 3480 (OH), 2960, 2925, 2870, 2850 (CH), 1750 cm^{-1} (C=O); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS}=0.00$); 6.18 (d, J=6 Hz, 7-H), 5.62 (s, broad, 1-H), 5.42 (s, 5-H), 4.37 (dd, $J_{8,14}=12$ Hz, $J_{7,8}=6$ Hz, 8-H), 4.03 (s, broad, 20-H_2, 3-H), 1.83 (s, 19-H_3), 3.32, 2.0-2.8 ppm (OH, exchangeable). As compared to Cy_{14} (**1a**), the main difference in the ^{1}H-NMR-spectrum are the paramagnetic shifts of 7-H and 8-H and the diamagnetic shifts of 1-H and 20-H_2. In contrast to the spectra of Cy_{14} and Cy_{12} (chart 1), 7-H and 8-H appear as a well resolved doublet and a doublet of doublets, respectively. The same differences were found in the 3-O- and 5-O-acylingenol series[17,18]. Thus, Euphorbia factor Cy_{13} is the 5-O-(2,3-dimethylbutyryl-13-O-*iso*-dodecanoyl-13-hydroxyingenol (**1b**).

Euphorbia factor Cy_{11} (table, **1d**) exhibits MS: 616 (M^+), 598 (M^+-18), 500 (M^+-116), 426 (M^+-190), 310 (base peak). The molecular formula, obtained by high resolution mass spectrometry, is $C_{36}H_{56}O_8$. The IR and UV-spectra are very similar to those of Cy_{14}, The ^{1}H-NMR-spectrum differs only in the number of protons at $\delta=1.25$ ppm. According to these data, Cy_{11} is the 3-O-(2,3-dimethylbutyryl)-13-O-*iso*-decanoyl-13-hydroxyingenol (**1d**). It is an isomer of TPA (12-O-tetradecanoylphorbol-13-acetat), the standard tumor promotor of mouse skin isolated from Crotonoil[14].

Tri-O-acylates of the polyfunctional diterpene parent 13,19-dihydroxyingenol (**2**). The 2nd group of diterpene esters comprises 3 compounds which show R_f-values similar to those of the Di-O-acyl-13-hydroxyingenols but which are different in partition behaviour and staining with vanillin/sulfuric acid spray reagent. Euphorbia factor Cy_6 (table, **2a**): MS: 674 (M^+), 656 (M^+-18), 558 (M^+-116), 540 (M^+-134), 442 (M^+-232), 424 (M^+-250), 326 (M^+-348, base peak), 308 (M^+-366); IR (CH_2Cl_2): ν_{max}: 3580, 3550, 3500, 3430 (OH), 2960, 2925, 2870 (CH), 1725 cm^{-1} (C=O); UV (CH_3OH): $\lambda(\varepsilon)$: 193 nm (17830); $\lambda_{max}(\varepsilon)$: 285 nm (290); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS}=0.00$; see also chart 2): 6.32 (s, broad, 1-H), 6.01 (d, broad, J=4 Hz, 7-H), 5.64 (s, 3-H), 4.63 (s, broad, 19-H_2), 4.15 (s, broad, 20-H_2), 4.1 (mc,

Structures and irritant doses 50 (ID_{50}) on the mouse ear[14] of diterpene esters isolated from *Euphorbia cyparissias* L.



1 (R^1-R^5=H)
2 (R^1-R^4=H, R^5=OH)

Euphorbia factor or compound	Molecular weight	R^1	R^2	R^3	R^4	R^5	ID_{50}* [nmoles/ear]
Cy_{14} (**1a**)	644	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	H	H	$COC_{11}H_{23}$	H	0.012
Cy_{13} (**1b**)	644	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	H	$COC_{11}H_{23}$	H	0.06
Cy_{12} (**1c**)	644	H	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	$COC_{11}H_{23}$	H	> 100
Cy_{11} (**1d**)	616	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	H	H	COC_9H_{19}	H	0.006
Cy_6 (**2a**)	674	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	H	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	OCO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	0.03
Cy_4 (**2b**)	674	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	OCO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	0.4
Cy_2 (**2c**)	674	H	H	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	CO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	OCO–CH(CH$_3$)–CH(CH$_3$)–CH$_3$	> 100

* Croton oil factor A_1 (12-O-tetradecanoylphorbol-13-acetat, TPA)[14] 0.016. Euphorbiafactor L_5 (3-O-hexadecanoylingenol)[15] 0.14.

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