On the Active Principles of the Euphorbiaceae, IX[a]
Ingenane Type Diterpene Esters from Five Euphorbia Species

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Investigations of *E. antiquorum, E. helioscopia, E. palustris, E. peplus* and *E. quadrialata* for irritant and tumor promoting constituents afforded several new ingenane type diterpene esters derived from the parent alcohols ingenol and 20-deoxyingenol and from the hitherto unknown 20-deoxy-16-hydroxyingenol and 20-deoxy-13.16-dihydroxyingenol. The irritant activities of the natural compounds are reported together with some aspects on structure activity relationships.

Introduction

The plant family Euphorbiaceae consists of about 290 genera. Among them, the largest genus Euphorbia comprises about 1600 species [1], occurring as succulent or non-succulent plants in most parts of the world. Plant parts of E. species are well known to be irritant. They are often used in folk medicine against all kinds of diseases [*e.g.* 2–4]. Many E. species growing wide-spread as weeds are held responsible for poisoning of live-stock [5].

During the last 15 years, in investigations of the chemical nature of the toxic principles of Euphorbiaceae for obvious reasons preferentially the latices of many of the large succulent and perennial Euphorbia species indigenous to Africa were used. They were shown to contain highly irritant and frequently tumor promoting diterpene esters of the tigliane, ingenane and daphnane type (for reviews see [6–8]). Only few investigations were carried out with herbaceous E. species indigenous to Europe occurring mostly as weeds. From European species, as active constituents so far esters of ingenol and of 16-hydroxyingenol have been isolated from *E. lathyris* [9] and *E. esula* [10] and esters of 13-hydroxyingenol and 13,19-dihydroxyingenol from *E. cyparissias* [11]. Moreover, esters of 20-deoxyingenol and 4-deoxyphorbol were obtained from *E. biglandulosa* [12] and esters of 12-deoxyphorbol from *E. helioscopia* [13].

We now report on the isolation of new ingenane type diterpene esters from the three European species *E. helioscopia, E. palustris* and *E. peplus*. Some of their esters or parent alcohols are identical with those isolated for comparison from the African succulent *E. quadrialata* and from the Indian succulent *E. antiquorum*. The active principle of a common Thai drug derived from *E. antiquorum* was described elsewhere [14].

Results

From *E. peplus* and *E. quadrialata* extracts of the latices, from the other plants extracts of the aerial parts were investigated. The constituents of all extracts were isolated in separation procedures using multiplicative distributions and chromatographic methods monitored by the standard assay for irritant activity on the mouse ear [15]; for further experimental details see also [16]. All factors ($ID_{50} < 50$ nmoles/ear) and compounds (practically non-irritant, $ID_{50} > 50$ nmoles/ear) are characterized with the first or the first two letters of the plant species from which they were isolated (letters for factors in bold-faced type). Their structures and irritant activities are summarized in the Table.

1. *Derivatives of ingenol* (**1**, Fig. 1)

From the latex of *E. antiquorum* and from a Thai purgative drug derived from it, Euphorbia factor $\mathbf{An_1}$ was isolated and characterized as 3-O-angeloylingenol (**2**). It forms readily the 5.20-O-isopropylidene derivative **3** upon treatment with acetone/*p*-toluene sulfonic acid hydrate [14]. Preparation of the acetonide from a crude irritant fraction obtained from stems of *E. antiquorum*, containing i.a.

[a] VIII[th] Communication, see: E. H. Seip, H. H. Ott, and E. Hecker, Planta Med. **49**, 199 (1983).
[b] Part of dissertation, see l. c. [16].
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Table. Structure and irritant activities (ID_{50}^{5} and ID_{50}^{24}) of factors or compounds isolated from three European and two Indian/African Euphorbia species; standards: TPA (12-O-tetradecanoylphorbol-13-acetate), ID_{50}^{5}: 0.010 nmoles/ear, ID_{50}^{24}: 0.016 nmoles/ear [29] and 3-TI (3-O-tetradecanoylingenol), ID_{50}^{24}: 0.011 nmoles/ear [18].

Factor/ compound	Parent alcohol	Acid moiety	ID_{50}^{5} [a] (nmoles/ear)	ID_{50}^{24} [a] (nmoles/ear)
H_1 (=An_1) (2)	ingenol	3-angelate	0.04	0.12
H_2, H_3 (8, 9)	ingenol	3-decatrienoate, 3-dodecatetraenoate	0.06	0.22
H_5 (10a)	ingenol	3-decadienoate	0.03	0.06
H_6 (10b)	ingenol	3-decadienoate	0.08	0.08
Pe_1 (12)	ingenol	20-acetate-3-angelate	n.d.	0.09
Pe_2 (=H_8) (17)	20-deoxyingenol	3-angelate	0.17	0.48
H_4 (18)	20-deoxyingenol	5-angelate	10	> 100
H_7 (20)	20-deoxyingenol	5-decatrienoate	9.0	> 100
P_2 (19)	20-deoxyingenol	5-benzoate	4.1	> 100
P_7 (23)	20-deoxyingenol	5-hexanoate	20	> 100
P_8 (24)	20-deoxyingenol	3,5-dibenzoate	> 100	> 100
P_9 (25)	20-deoxyingenol	5-benzoate-3-hexanoate	> 100	> 100
Q_1 (30)	20-deoxy-16-hydroxyingenol	16-acetate-3-angelate	n.d.	12.0
P_6 (33)	20-deoxy-16-hydroxyingenol	3,16-dibenzoate	2.5	5.0
P_1 (32)	20-deoxy-16-hydroxyingenol	5,16-dibenzoate	> 100	> 100
P_3 (40)	20-deoxy-13,16-dihydroxyingen.	3,16-dibenzoate-13-phenylacetate	> 50	> 50
P_4 (41)	20-deoxy-13,16-dihydroxyingen.	13-acetate-3,16-dibenzoate	> 50	> 50
P_5 (39)	20-deoxy-13,16-dihydroxyingen.	3,13,16-tribenzoate	n.d.	18

n.d. = not determined.
[a] Standard deviation σ: 1.3; level of significance $\alpha = 0.05$.

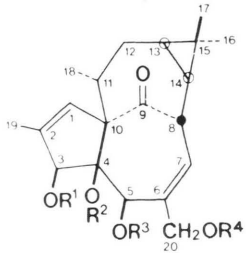


Fig. 1. Structure of ingenol (**1**) and its derivatives **2**–**12.**

1: R^1–R^4 = H
2: R^1 = angeloyl, R^2–R^4 = H (**An_1 = H_1**)
3: R^1 = angeloyl, R^2 = H, R^3, R^4 = isopropylidene
4: R^1 = R^2 = H, R^3, R^4 = isopropylidene
5: R^1, R^2 = R^3, R^4 = isopropylidene
6: R^1 = $CO(CH{=}CH)_3{-}(CH_2)_2{-}CH_3$, R^2 = H, R^3, R^4 = isopropylidene
7: R^1 = $CO(CH{=}CH)_4{-}(CH_2)_2{-}CH_3$, R^2 = H, R^3, R^4 = isopropylidene
8: R^1 = $CO(CH{=}CH)_3{-}(CH_2)_2{-}CH_3$, R^2–R^4 = H (**H_2**)
9: R^1 = $CO(CH{=}CH)_4{-}(CH_2)_2{-}CH_3$, R^2–R^4 = H (**H_3**)
Z/E
10a,b: R^1 = $CO(CH{=}CH)_2{-}(CH_2)_4{-}CH_3$, R^2–R^4 = H (**H_5** and **H_6**)
Z/E
11a,b: R^1 = $CO(CH{=}CH)_2{-}(CH_2)_4{-}CH_3$, R^2 = H, R^3, R^4 = isopropylidene
12: R^1 = angeloyl, R^2 = R^3 = H, R^4 = $COCH_3$ (**Pe_1**)

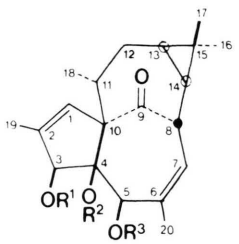
chlorophyll, proved useful for isolation and characterisation of **An_1** as **3,** which could easily be separated by TLC.

A more refined separation procedure with *E. helioscopia* (for details see [16]) afforded several factors (**H_1–H_3, H_5, H_6**), characterized as 3-acylates of ingenol (**1**) with unsaturated acid moieties. According to spectral data and chromatographic properties factor **H_1** is identical with **An_1.** Again, **H_1** furnished the isopropylidene derivative **3**, which upon transesterification with sodium methanolate afforded 5.20-O-isopropylideneingenol **4** [17]. Transesterification of the mixture **H_2, H_3** not separable by TLC (silica gel) with 0.1 M $NaOCH_3$/MeOH yielded ingenol (characterized as its 3.4:5.20-di-O-isopropylidene derivative **5** [17]) and unsaturated methyl esters showing UV absorbance at 346 (tetraenoate) and 307 nm (trienoate). After catalytic hydrogenation they were identified by GLC analysis as methyl decanoate and dodecanoate. **H_2, H_3** afforded the mixture of acetonides **6, 7** with a sharp singlet at 5.60 ppm (3-H) in the NMR spectrum. Thus **H_2** represents 3-O-(2.4.6-decatrienoyl)ingenol (**8**) and **H_3** 3-O-(2.4.6.8-dodecatetraenoyl)ingenol (**9**). Factors **H_5**

and **H_6** seem to be geometric isomers of 3-O-(2.4-decadienoyl)ingenol (**10a,b**). Differences in the chemical shifts in the NMR spectra of the acetonides **11a** and **11b** show up only in the region of $\delta > 5.6$ ppm. After transesterification of **11a** and **11b** and catalytic hydrogenation of the resulting methyl esters in both cases decanoic acid methyl ester was identified as the major compound by GLC.

Factor **Pe_1** from E. peplus (M^+ at *m/e* 472) shows similar UV data as **An_1**. In the NMR spectrum an additional singlet of an acetyl group appears at 2.09 ppm and the signal for 20-H_2 at 4.65 ppm. It was shown by selective acetylation [18] of **An_1** that factor **Pe_1** represents 20-O-acetyl-3-O-angeloylingenol (**12**)*. A compound with the same structure, tentatively assigned, was isolated from Euphorbia kamerunica [21].

Fig. 2. Structures of 20-deoxyingenol (**13**) and its derivatives **14–25.**



13: $R^1-R^3 = H$
14: $R^1 = R^3 = COCH_3$, $R^2 = H$
15: R^1, R^2 = isopropylidene, $R^3 = H$
16: R^1, R^2 = isopropylidene, R^3 = angeloyl
17: R^1 = angeloyl, $R^2 = R^3 = H$ (**H_8 = Pe_2**)
18: $R^1 = R^2 = H$, R^3 = angeloyl (**H_4**)
19: $R^1 = R^2 = H$, R^3 = benzoyl (**P_2**)
20: $R^1 = R^2 = H$, $R^3 = CO(CH{=}CH)_3-(CH_2)_2-CH_3$ (**H_7**)
21: R^1, R^2 = isopropylidene, R^3 = benzoyl
22: R^1, R^2 = isopropylidene, $R^3 = CO(CH{=}CH)_3-(CH_2)_2-CH_3$
23: R^1, $R^2 = H$, $R^3 = COC_5H_{11}$ (**P_7**)
24: $R^1 = R^3$ = benzoyl, $R^2 = H$ (P_8)
25: $R^1 = COC_5H_{11}$, $R^2 = H$, R^3 = benzoyl (P_9)

* A recent report [19] describes the isolation of a compound from Euphorbia canariensis which was assigned the structure of the isomeric 3-O-acetyl-20-O-angeloylingenol. The spectral data are identical with those of **Pe_1**. Positioning of the acid moieties [19] was deduced from a selective hydrolysis reaction affording 20-O-angeloylingenol. Under such reaction conditions (0.1 M KOH), however, migration of the acyl residues in ingenol esters from O-3 to O-20 are well known [18, 20]. Hence, the 20-angelate could have been generated from a factor identical with **Pe_1** by base catalyzed rearrangement of the primary product of hydrolysis, *i. e.* 3-O-angeloylingenol.

2. Derivatives of 20-deoxyingenol (**13,** Fig. 2)

Three further ingenane type angelates with a molecular weight of 414 are Euphorbia factors **H_4, H_8** and **Pe_2. Pe_2** and **H_8** show the same spectroscopic and chromatographic properties and are therefore identical. Their NMR spectra differ from that of **An_1** mainly by the absence of the signal of 20-H_2, whereas an additional broad singlet of a methyl group appears at 1.80 ppm superimposed with that of 19-H_3. Decoupling experiments establish this signal to indicate a 20-H_3 group. This assignment also explains the diamagnetic shift of protons 5-H and 7-H of about 0.3 ppm. Transesterification of **Pe_2** afforded the crystalline parent compound 20-deoxyingenol (**13**) [22], which was characterized as the 3,5-diacetate and the 3.4-O-isopropylidene derivative **15.**

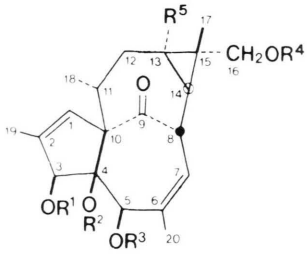
The difference between **H_8** and **H_4** is revealed by the NMR spectra. The sharp singlet at 5.45 ppm is consistent with the presence of a 3-acylate for **H_8.** In the spectrum of **H_4** this signal is replaced by a broad singlet at 5.25 ppm similar to 5-acylates in the ingenol series [18, 20]. An additional support for these assignments was given by treating both factors with acetone/*p*-toluenesulfonic acid hydrate. Only **H_4** afforded an acetonide **16.** Therefore **H_8** and **Pe_2** represent the 3-angelate **17** and **H_4** the 5-angelate **18** of 20-deoxyingenol (**13**).

Two further Euphorbia factors were identified as 5-acylates of **13. P_2** was characterized as the benzoate **19, H_7** as the 2.4.6-decatrienoate **20.** In both cases position of the acid moieties were ensured by preparing the acetonides **21** and **22,** respectively. Transesterification of **H_7** yielded 20-deoxyingenol (**13**) and a corresponding unsaturated methyl ester showing UV absorbance at 302 nm. After catalytic hydrogenation it exhibited an identical retention time as methyl decanoate. Euphorbia factor **P_7** must be a 5-hexanoate **23** according to the mass spectrum (M^+ at *m/e* = 430) and the NMR spectrum (5-H at 5.20 ppm). As indicated by NMR the acid residue is probably branched.

Also two 3.5-diesters of **13,** compounds P_8 and P_9, were isolated from E. palustris. P_8 shows spectral data identical with those of the 3.5-dibenzoate **24** prepared from **13** by treatment with benzoyl chloride/pyridine. P_9 is a hexanoate-benzoate with a probably branched aliphatic chain like in **P_7.** In the NMR spectrum of P_9 a diamagnetic shift for the signals of 1-H (0.16 ppm) and 3-H (0.32 ppm) compared to those in the spectrum of P_8 is observed. This may indicate

that 3-OH in P_9 is esterified with the aliphatic acid, *i. e.* compound P_9 represents structure **25.**

Fig. 3. Structure of 20-deoxy-16-hydroxyingenol (**26**) and its derivatives **27–36** and of 20-deoxy-13,16-dihydroxyingenol (**37**) and its derivatives **38–41.**



26: R^1–R^5 = H
27: R^1 = angeloyl, R^2–R^5 = H
28: R^1 = R^2 = R^4 = R^5 = H, R^3 = angeloyl
29: R^1 = R^3 = R^4 = $COCH_3$, R^2 = R^5 = H
30: R^1 = angeloyl, R^2 = R^3 = R^5 = H, R^4 = $COCh_3$ (**Q_1**)
31: R^1, R^2 = isopropylidene, R^3 = R^4 = benzoyl, R^5 = H
32: R^1 = R^2 = R^5 = H, R^3 = R^4 = benzoyl, (P_1)
33: R^1 = R^4 = benzoyl, R^2 = R^3 = R^5 = H (**P_6**)
34: R^1–R^3 = R^5 = H, R^4 = benzoyl
35: R^1, R^2 = isopropylidene, R^3 = R^5 = H, R^4 = benzoyl
36: R^1, R^2 = isopropylidene, R^3–R^5 = H
37: R^1–R^4 = H, R^5 = OH
38: R^1 = R^4 = benzoyl, R^2 = R^3 = H, R^5 = benzoyloxy (**P_5**)
39: R^1 = R^4 = benzoyl, R^2 = R^3 = H, R^5 = $OCOCH_2C_6H_5$ (P_3)
40: R^1 = R^4 = benzoyl, R^2 = R^3 = H, R^5 = $OCOCH_3$ (P_4)
41: R^1 = R^2 = H, R^3 = R^4 = benzoyl, R^5 = $OCOCH_3$

3. Derivatives of 20-deoxy-16-hydroxyingenol (**26,** Fig. 3)

Euphorbia factor **Q_1,** an angelate-acetate was isolated from *E. quadrialata.* The chemical shifts of the signals in the NMR spectrum, however, are similar to those in the spectrum of **H_8.** Decoupling experiments give proof for a 20-H_3 group; furthermore an AB-system appears at 4.16 ppm, which is due to a hydroxymethyl group in the ingenane skeleton. The appearance of only one singlet of a cyclopropylic methyl group and the provable doublet of 18-H_3 at 0.95 ppm suggest this position to be at C-16 or C-17. Like in derivatives of 16-hydroxyingenol [23] an evident shifting of the signal of 14-H to lower field can be shown by decoupling experiments. Whereas in **H_8** this signal appears at 0.85 ppm it is now provable at 1.08 ppm. So it may be deduced that the oxygenation has occurred at C-16. The parent compound 20-deoxy-16-hydroxyingenol (**26**) was shown to be diterpene parent alcohol of esters occuring in *E. marginata* [24].

The positions of the different acid residues in **Q_1** were investigated by mild base catalyzed transesterification. Treatment with 5×10^{-3} M $NaOCH_3$/MeOH afforded the 3-angelate **27.** In a smaller amount, the 5-angelate **28** was obtained probably formed by acyl migration as known for ingenol-3-acylates [18]. In about the same amount also the parent alcohol **26** was isolated and characterized as the 3.5.16-triacetate **29.** Hence, spectral data and chemical reactions prove structure **30** for Euphorbia factor **Q_1.**

26 was also shown to be the parent alcohol of two dibenzoates, factor **P_6** and compound P_1. An AB-system at 4.46 ppm in the NMR spectrum of **P_6** and a broad singlet at 4.55 ppm in that of P_1 indicate benzoylation of the primary hydroxyl group in both cases. The other ester positions are distinguishable like in the series of 20-deoxyingenol. Whereas **P_6** shows a sharp singlet at 5.73 ppm, indicating 3-H and superimposed with the multiplet of 7-H, P_1 has a comparable but broader singlet at 5.46 ppm, which must be due to the proton 5-H. Formation of the acetonide **31** from P_1 is an additional evidende that P_1 represents the 5,16-dibenzoate **32** since only this substitution type (out of six possible diesters) should afford an acetonide according to the Dreiding-Model. Factor **P_6** therefore is the isomeric 3,16-dibenzoate **33.**

The dibenzoates P_1 (**32**) and **P_6** (**33**) gave the 16-benzoate **34** after short treatment (1 h) with 0.1 M $NaOCH_3$/MeOH. **34** afforded the isopropylidene derivative **35,** which upon transesterification furnished **36.** Complete transesterification of P_1 or **P_6** occurred within 24 h to yield the parent alcohol **26** which was characterized as its 3.5.16-triacetate **29.**

4. Derivatives of 20-deoxy-13.16-dihydroxyingenol (**37,** Fig. 3)

Three other constituents of *E. palustris,* compounds P_3 and P_4 and factor **P_5,** exhibit strikingly similar NMR spectra like factor **P_6** concerning the characteristic signals between 3 and 7 ppm. Combined with the mass spectra, however, they give evidence for triesters of a parent compound, which differs from **26** by an additional oxygen atom. **P_5** (M^+ at m/e = 676) shows signals of 15 aromatic protons in the NMR which indicate a tribenzoate. Because no new signal in the spectrum can be observed the addi-

tional oxygen atom should be attached to a tertiary C-atom in the ingenane skeleton. The position is revealed by decoupling experiments. By irradiation at 4.3 ppm (8-H) the doublet at 1.61 ppm (J = 12 Hz) is changed to a sharp singlet. This low field shift for 14-H and the signals of the apparently more deshielded protons 11-H and 12-H_2 indicate that the oxygenation must have occurred at C-13. From these data and comparison with factor **P_6**, **P_5** can be regarded as the 3.13.16-tribenzoate **38** of the new 20-deoxy-13.16-dihydroxyingenol (**37**). The spectral data of P_3 and P_4 prove them to be a dibenzoate-phenylacetate **39** and a dibenzoate-acetate **40**, respectively, of the same parent alcohol. Probably the acid moieties differing from those in **P_5** are located at C-13, because the chemical shifts of 3-H and 16-H_2 have not changed.

Chromatographic investigations suggest an instability of these compounds. After storage of P_4 for six months in ethyl acetate the corresponding 5.13.16-triester **41** was isolated in a yield of about 30%. Obviously similar acyl migrations occur as in the series of ingenol-3-acylates [18].

Discussion

Esters of ingenol have been isolated from many species of Euphorbiaceae in recent years (for review see [6–8]). From the three European Euphorbia species investigated here further esters of ingenol (**1**), 20-deoxyingenol (**13**), 20-deoxy-16-hydroxyingenol (**26**) and 20-deoxy-13.16-dihydroxyingenol (**37**) were obtained (for overview see Table). The same or similar esters of ingenol and 20-deoxy-16-hydroxyingenol were shown to be present in *E. antiquorum* indigenous to India and in the African species *E. quadrialata*, respectively.

In our investigation of *E. helioscopia* the presence of 12-deoxyphorbol esters as reported by Evans *et al.* [13] was not confirmed. The reason for this may be the difference in origin of the plant specimens investigated: it is known, that *e.g.* latex from *E. tirucalli* collected in Africa compared with latex collected in Madagascar shows quantitatively different pattern of diterpene esters of the tigliane and ingenane type [25, 26]). Perhaps the biosynthetic routes producing these irritant diterpene esters depend on soil and/or climate where these plants are grown.

Nearly all ingenane type factors isolated exhibit their highest irritant activity on the mouse ear already 5 hours after application (see Table), whereas 3-O-tetradecanoylingenol (3-TI), as a standard, shows its maximum 24 h after application [18]. Among all factors isolated, 3-esters of *ingenol* exhibit the highest irritant activity (see Table), though all of them are clearly less active than 3-TI after 24 h. Unsaturation of the acid moieties, either in long chain carboxylic acids (C_{10} in factors **H_2**, **H_5** and **H_6** and C_{12} in factor **H_3**) or in short chain acids (angelic acid in factor **H_1** or **An_1**) reduces the exposure time to reach the maximum of the irritant activity (*i.e.* minimum of ID_{50}) at 5 h. When **H_1** is esterified in 20-position with acetic acid (as in **Pe_1**) the activity measured 24 h after application on the mouse ear is comparable (ID_{50}^{5} value was not determined).

3-Esters of *20-deoxyingenol* still exhibit considerable irritant activity. Compared to 3-O-angeloylingenol, 3-O-angeloyl-20-deoxyingenol (**Pe_2** or **H_8**) is about four times less active. With phorbol-12.13-diesters it was found that presence of a 20-hydroxyl group is a prerequisite for irritant activity [27]. 5-Esters of 20-deoxyingenol (**H_4**, **H_7** and **P_2**, **P_7**) are only weak irritants 5 h after application (ID_{50}^{5} between 4 and 20 nmoles/ear) and practically inactive 24 hours after application on the mouse ear ($ID_{50}^{24} > 100$ nmoles/ear). The 3,5-diesters of the same parent alcohol, P_8 and P_9, are inactive.

Whereas 3.16-diesters of 16-hydroxyingenol are highly active as irritants [23], 3.16-diesters of the new parent alcohol *20-deoxy-16-hydroxyingenol* (**P_6** and **Q_1**) only exhibit weak irritant activity, and the 5.16-diester P_1 of the same parent is practically non-irritant.

The 3.13.16-tribenzoate of the new parent alcohol *20-deoxy-13.16-dihydroxyingenol* (**P_5**) is a weak irritant, too, and the aromatic/aliphatic 3.13.16-triesters, P_3 and P_4, are non-irritant. On the other hand it is known that 3.13.19-triesters of 13.19-dihydroxyingenol are highly irritant [11].

3-Angelates of ingenol and of 20-deoxyingenol were previously also isolated from latex of *E. paralias* [28]. They were tested for irritant activity, but the ID_{50} values obtained are not comparable with the present data, perhaps due to use of different strains of mice. Similarly as with factors **H_1** and **H_8**, they exhibited 2 h after application on the mouse ear higher irritant activity than at 24 h. However, 3-O-angeloyl-20-deoxyingenol showed a *ca.* 20 fold higher activity than 3-O-angeloylingenol, which is differing considerably from our data.

Experimental

IR spectra were measured with a Perkin-Elmer spectrometer model 521, the UV spectra (in methanol) with a Beckman spectralphotometer Acta M VI, the CD spectra with a dichrograph of Jobun Yvon, Paris. Mass spectra were obtained with a Varian spectrometer MAT 711. NMR spectra were run in $CDCl_3$ (unless otherwise stated) with instruments from Bruker Physik AG, models HX-90 and MH-90. Tetramethylsilane was used as an internal standard (δ = 0.00 ppm). OH-protons were detected by exchange with D_2O.

For analytical and preparative TLC silica gel MN P/UV_{254} from Macherey & Nagel, Düren, was used as well as silica gel plates 60 F_{254} from E. Merck, Darmstadt. Columns were prepared with silica gel 0.063 – 0.2 mm from Woelm, Eschwege. For GLC-analyses a Becker-Packard chromatograph model 420 was employed with a column of 10% GESE on chromosorb W/HP 80/100. Sports on TLC were visualized with vanillin-sulfuric acid.

Solvent systems used for *multiplicative distributions:* system A = petroleum ether/methanol/water 15/10/0.5; system B = tetrachloromethane/methanol/water 4/2/0.16. Machinery and methods are described elsewhere [15].

The separation procedures were accompanied by a standardized test for irritant activity on the mouse ear [15].

Plant materials. 1 kg of plant stems of *E. antiquorum* were purchased from F. G. Celo, Medicinal Plants Inc., Zweibrücken. Latex of *E. peplus* was collected in July and August in vineyards near Heidelberg. The stalks were cut below the umbel and the latex collected with glass capillary tubes. These were crushed under acetone and the material was extracted several times with acetone. Aerial parts of *E. helioscopia* and *E. palustris* were purchased from Heinrich Bornträger, Drugs and Spices, Offstein (Germany). After collection all plant materials were cut and preserved and stored under methanol. The methanolic liquid of plant preparations was combined with the methanol extracts (see below) of corresponding plant parts. A latex preparation of *E. quadrialata* in methanol was provided by the late Dr. P. R. O. Bally, Nairobi, Kenya.

Separation procedures

I. Aerial Plant parts

E. antiquorum. 1 kg of green stems of *E. antiquorum*, preserved under methanol were homogenized with an „Ultra Turrax" and extracted exhaustively with methanol to yield 16.7 g of a methanol extract. Partitioning between water and ethyl acetate afforded 2.75 g of an ethyl acetate extract (ID_{50}: 1.5 μg/ear). 1 g of this extract was separated by prep. TLC in ether/petroleum ether = 4/1 and 30 mg of a crude irritant fraction were obtained, still not homogeneous according to TLC. This fraction was treated with acetone and catalytic amounts of *p*-toluene sulfonic acid hydrate for 30 min. After purification of the reaction product by prep. TLC in ether/petroleum ether = 1/1 3.0 mg of an isopropylidene derivative were obtained, R_f (ether/petroleum ether = 1/1): 0.4; all spectral data are identical with those described for compound **3** (see below).

E. helioscopia. 12 kg of aerial parts of *E. helioscopia* yielded 923 g of a methanol extract. Water and ethyl acetate were added and the aqueous layer was extracted with ethyl acetate for several times. The ethyl acetate extract obtained (157 g, ID_{50} 29 μg/ear) was distributed successively according to O'Keeffe in the solvent systems A and B. The remaining irritant active fraction (27.4 g, ID_{50} 6.4 μg/ear) was subjected to a Craig distribution in system A with n = 4000 transfers. Then irritant principles were indicated in the fractions of G = 0.06–0.18. From these fractions by means of prep. TLC the Euphorbia factors **H_1, H_4–H_8** and the mixture of Euphorbia factors **H_2, H_3** were isolated.

E. palustris. In an equivalent procedure irritant principles were concentrated from the methanol extract (883 g) from 11.3 kg of *E. palustris.* The ethyl acetate extract weighed 154 g. After two O'Keeffe distributions the active fraction (16.0 g, ID_{50} 5.7 μg/ear) was subjected to a Craig distribution with n = 3000 transfers. Irritant principles were found within the fractions showing G = 0.05–0.11. From these fractions the Euphorbia factors **P_2** and **P_5–P_7** were isolated besides the compounds P_1, P_3, P_4. Further similar compounds, P_8 and P_9 were isolated from more hydrophobic non-irritant fractions which had been separated after the second O'Keeffe distribution.

II. Latices

E. antiquorum (see [14])

E. peplus. 380 mg of an acetone extract of latex (ID_{50} 0.85 μg/ear) was distributed in system A with n = 35 transfers according to Craig. From the hydrophilic fraction (199 mg, ID_{50} 0.38 μg/ear) the Euphorbia factors **Pe_1** and **Pe_2** could be isolated by means of prep. TLC.

E. quadrialata. Extraction of the latex preparation (100 g) with acetone yielded 20 g of acetone soluble material (IU: 6.6 μg/ear). By a Craig distribution using system A the acetone extract was separated into

the non-irritant hydrophobic fraction (IU: >2000 μg/ear) and the irritant hydrophilic fraction (2.5 g; ID_{50}: 2.2 μg/ear). From this fraction factor **Q_1** (23 mg) was isolated using prep. TLC.

Chemical reactions

Transesterification. About 10 mg of a sample were treated with 1 ml of 0.1 M $NaOCH_3$/MeOH for 1–5 h at room temperature. After addition of phosphate buffer, pH 6.7, the solution was extracted with ethyl acetate. The organic layers were dried with $MgSO_4$, and after evaporation of the solvent the products were isolated by prep. TLC in suitable solvent systems.

Preparation of isopropylidene derivatives. The sample was dissolved in a small volume of acetone and then treated with a catalytic amount of *p*-toluene sulfonic acid hydrate for 1–24 h at room temperature. The reaction was stopped by adding phosphate buffer pH 6.7, the products were extracted with ethyl acetate and purified by prep. TLC.

Acetylations. About 10 mg of a sample was dissolved in 5 ml of pyridine/acetic anhydride 3/1 and kept at room temperature for 12–24 h. After hydrolization with phosphate buffer pH 6.7, the products were extracted with ethyl acetate. The organic layer was washed with 1 M HCl then with buffer and finally with water. After drying ($MgSO_4$) and evaporation of the solvent, purification was carried out by prep. TLC.

Hydrogenations of unsaturated fatty acid methyl esters. The transesterification products (usually 2 mg) were dissolved in about 5 ml of ethyl acetate. After adding a catalytic amount of palladium-charcoal the reaction mixture was stirred for 2–3 h under hydrogen. At the end of the reaction the catalyst was removed by filtration.

1. Derivatives of ingenol (**1**)

Euphorbia factor **An_1** (**2**, data see l. c. [14])

Euphorbia factor **H_1** (**2**)

R_f (petroleum ether/ethyl acetate 1/1): 0.28. MS: *m/e* = 430 (M^+), 330, 312. IR (CH_2Cl_2): 3500 (OH), 1718 (CO), 1642 cm^{-1} (C=C). UV: λ (ε) = 194 nm (18500); λ_{max} (ε) = 211 (16700), 296 nm (370). ^1H NMR: 1-H, 7-H: 6.05 (m); 3-H: 5.53 (s), 20-H_2: 4.14 (s); 5-H: 4.05 (s, br); 8-H: 4.2–4.0 (superimposed); 19-H_3: 1.80 (d, *J* = 1.5 Hz); 16-H_3, 17-H_3: 1.06 (s) and 1.09 (s); 18-H_3: 0.96 (d, *J* = 7 Hz); OH: 3.52 ppm; angelate: 1 olefinic H: 6.15 (m); 2 olefinic CH_3: 2.1–1.9 ppm (m). ^{13}C NMR: 207.45 (s); 168.65 (s); 139.86 (s); 139.27 (d); 136.15 (s); 132.12 (d); 127.84 (d); 127.64 (s); 85.07 (s); 82.47 (d); 76.04 (d); 71.81 (s); 66.87 (t); 43.48 (d); 38.41 (d); 31.00 (t); 28.53 (q); 24.11 (s); 23.27 (d); 22.94 (d); 20.80 (q); 17.09 (q); 15.92 (q); 15.60 ppm (2 q).

a) Preparation of the acetonide **3** of factor **H_1:** R_f (petroleum ether/ether 1/1): 0.4. MS: *m/e* = 470 (M^+). UV: $\lambda(\varepsilon)$ = 194 nm (19000); λ_{max} (ε) = 209 (16800), 298 nm (400). ^1H NMR: 1-H: 6.05 (m); 7-H: 5.75 (m); 3-H: 5.63 (s); 20-H_2: 4.18 (s); 8-H: 4.2–4.0 (superimposed); 5-H: 4.0 (s, br.); 19-H_3: 1.8 (d, *J* = 1.5 Hz); 16-H_3, 17-H_3: 1.09 (s) and 1.04 (s); 18-H_3: 0.97 (d, *J* = 8 Hz); OH: 3.18 ppm; angelate: 1 olefinic H: 6.1–6.0 (m); 2 olefinic CH_3: 2.1–1.9 ppm; isopropylidene: 1.46 (s) and 1.42 ppm (s).

b) Transesterification of **3** affording ingenol-5,20-acetonide (**4**): All spectral data of **4** are identical with those described in [17].

Mixture of Euphorbia factors **H_2, H_3** (**8, 9**)

R_f (dichloromethane/acetone 4/1): 0.3–0.4. MS: *m/e* = 522 (M^+, **H_3**), 496 (M^+, **H_2**) 330, 312. ^1H NMR: 9 H (including 1-H, 7-H, 3-H and olefinic H): 8.0–5.5; 5-H, 8-H, 20-H_2: 4.2–4.0 (superimposed); 19-H_3: 1.80 (s, br.); *ca.* 10 H: 1.7–1.2; 16-H_3, 17-H_3: 1.09 (s) and 1.07 (s); OH: 3.73 (s); 3.55 (s); 2.6–2.3 ppm (superimposed).

a) Transesterification of factors **H_2, H_3** affording **1** and unsaturated methyl esters: Characterisation of **1** by preparation of the ingenol-3,4:5,20-diacetonide (**5**), spectral data identical with those described in [17]. Catalytic hydrogenation of the unsaturated methyl esters yielded methyl dodecanoate (rel. int. 30%), methyl decanoate (100%), others (<3%) as identified by GLC analysis with authentic references.

b) Preparation of the acetonides **6, 7** of factors **H_2, H_3:** MS: *m/e* = 562 (M^+), 536 (M^+). IR: 3520 (OH); 1712 (CO); 1608, 1590 cm^{-1} (C=C). UV: λ = 194 nm; λ_{max} = 312, 354 nm. ^1H NMR: 10 H (incl. 1-H, 7-H): 8.0–5.5; 3-H: 5.60 (s); 5-H, 8-H, 20-H_2: 4.25–4.0; 19-H_3: 1.79 (d, *J* = 1.5 Hz); 16-H_3, 17-H_3: 1.06 (s) and 1.04 (s); OH: 3.20 (s); isopropylidene: 1.47 (s) and 1.42 ppm (s).

Euphorbia factor **H_5** (**10a**)

R_f (dichloromethane/acetone 4/1): 0.36. MS: *m/e* = 498 (M^+), 330, 312.

a) Preparation of the acetonide **11a** of factor **H_5:** R_f (ether/petroleum ether 1/1): 0.5. MS: *m/e* = 538 (M^+). UV: λ (ε) = 194 nm (15040); λ_{max} (ε) = 266 (14130), 314 nm (sh, 1660). ^1H NMR: 1 olefinic H: 7.62 (dd, *J* = 15 and 10 Hz); 5 H (including 1-H, 7-H): 6.25–5.65; 3-H: 5.60 (s); 5-H, 8-H, 20-H_2: 4.2–4.0; 19-H_3: 1.78 (d, *J* = 1.5 Hz); *ca.* 7 H: 1.3

(m); 16-H_3, 17-H_3: 1.08 (s) and 1.04 (s); *ca.* 5 H: 1.00–0.65; OH: 3.18 (s); isopropylidene: 1.47 (s) and 1.42 ppm (s).

b) Transesterification of **11a** affording ingenol-5,20-acetonide (**4**) and unsaturated methyl esters: Spectral data of **4** were identical with those described in [17]. Catalytic hydrogenation of the unsaturated methyl esters yielded methyl decanoate (rel. int. 100%) and others (17%, 19%, 25%, <5%) as identified by GLC analysis with authentic references.

Euphorbia factor $\mathbf{H_6}$ (**10b**)

R_f (dichloromethane/acetone 4/1): 0.41. MS: *m/e* = 498 (M^+).

a) Preparation of the acetonide **11b** of factor $\mathbf{H_6}$: R_f (ether/petroleum ether 1/1): 0.5. MS: *m/e* = 480 (M^+-acetone). UV: λ = 269, 320 nm (sh). ^{1}H NMR: 1 olefinic H: 7.2–6.8 (m), 5 H (including 1-H and 7-H): 6.1–5.6 ppm. All other data as in the spectrum of **10b.**

b) Transesterification of **11b** affording ingenol-5,20-acetonide (**4**) and unsaturated methyl esters: **4** die cochromatograph with an authentic sample, staining: brown. Catalytic hydrogenation of the unsaturated methyl esters yielded methyl decanoate (rel. int. 100%) and others (13%, 44%, 13%, 14%, <10%) as identified by GLC analysis with authentic references.

Euphorbia factor $\mathbf{Pe_1}$ (**12**)

R_f (petroleum ether/ether 1/3): 0.31. MS: *m/e* = 472 (M^+), 312, 294. IR (CH_2Cl_2): 3550, 3550–3300, (OH, br.); 1716 (CO); 1640 cm^{-1} (C=C). UV (CH_2Cl_2): λ (ε) = 195 nm (14360); λ_{max} (ε = 212 (13800); 295 (310); 330 nm (190). ^{1}H NMR: 1-H, 7-H, 1 olefinic H: 6.2–6.0 (superimposed); 3-H: 5.60 (s); 20-H_2: 4.65 ± 0.13 (J_{AB} = 13 Hz); 8-H: 4.15 (m); 5-H: 3.93 (s, br.); 19-H_3: 1.83 (s, br.); 16-H_3, 17-H_3: 1.09 (s) and 1.07 (s); 18-H_3: 0.98 (d, *J* = 7 Hz); OH: 3.8 (br., 1 H); 3.58 ppm (s); acetate: 2.09; angelate: 2 olefinic CH_3: 2.09–1.97 ppm (m).

a) Partial synthesis of **12** from factor $\mathbf{An_1}$: 111 mg of $\mathbf{An_1}$ (**2**) were dissolved in 10 ml of dichloromethane/pyridine 4/1 and treated with 400 mg of acetic anhydride for 1 h at room temperature. Yield: 92 mg of **12** (76%). MS- and ^{1}H NMR data are identical with those described for $\mathbf{Pe_1}$.

2. Derivatives of 20-deoxyingenol (**13**)

Euphorbia factor $\mathbf{Pe_2}$ ($\mathbf{H_8}$) (**17**)

R_f (ether/petroleum ether 1/1): 0.30. MS: *m/e* = 414 (M^+), 314, 296. IR (CH_2Cl_2): 3560, 3510 (br., OH); 1712 (CO); 1639 cm^{-1} (C=C). UV: λ (ε) = 195 nm (14690); λ_{max} (ε) = 212 (14690), 280 nm (sh, 400). ^{1}H NMR: 1-H: 6.06 (d, *J* = 1.5 Hz); 7-H: 5.75 (m); 3-H: 5.45 (s); 8-H: 4.02 (m); 5-H: 3.69 (d, *J* = 7 Hz); 19-H_3, 20-H_3: 1.80 (s, br.); OH: 3.44 (s); 3.17 (d, *J* = 7 Hz); angelate: 1 olefinic H: 6.16 (m); 2 olefinic CH_3: 2.02–1.93 ppm (m); ^{13}C NMR: 207.06 (s); 168.71 (s); 139.47 (d); 137.58 (s); 136.57 (s); 132.71 (d); 127.51 (s); 124.07 (d); 85.07 (s); 83.19 (d); 77.08 (d); 71.88 (s); 43.41 (d); 38.73 (d); 31.07 (t); 28.53 (q); 24.05 (s); 23.33 (d); 23.14 (d); 21.97 (q); 20.73 (q); 17.04 (q); 15.86 (q); 15.53 (2 q) ppm.

a) Transesterification of factor $\mathbf{Pe_2}$ affording 20-deoxyingeol (**13**): M. p. 205–208 °C (from ethyl acetate). R_f (ether/petroleum ether 3/1): 0.26, staining: orange-brown. MS: *m/e* = 332 (M^+). UV: λ (ε) = 193 nm (14510); λ_{max} (ε) = 292 nm (237). ^{1}H NMR: 1-H: 5.97 (m); 7-H: 5.73 (m); 3-H: 4.40 (s); 8-H: 3.99 (m); 5-H: 3.41 (d, br., *J* = 9 Hz); 19-H_3: 1.86 (d, *J* = 1.5 Hz); 20-H_3: 1.78 (s, br.); 16-H_3, 17-H_3: 1.12 (s) and 1.07 (s); 18-H_3: 0.96 (d, *J* = 7 Hz); OH: 4.07 (d); 2.56 (d, *J* = 9 Hz); 2.3 ppm (superimposed). ^{1}H NMR (C_5D_5N): Chemical shifts and multiplicity are identical with those published by Uemura *et. al.* [22].

b) Preparation of the acetonide **15** of 20-deoxyingenol (**13**): R_f (petroleum ether/ether 3/2): 0.35. MS: *m/e* = 372 (M^+). IR (KBr): 3465 (OH); 1717 cm^{-1} (C=O). UV: λ (ε) = 193 nm (12750); λ_{max} (ε) = 292 nm (240). ^{1}H NMR: 1-H, 7-H: 5.80 (m, superimposed); 3-H: 4.67 (s); 8-H: 4.00 (m); 5-H: 3.78 (d, *J* = 8 Hz); 19-H_3, 20-H_3: 1.85 (m, superimposed); 16-H_3, 17-H_3: 1.16 (s) and 1.04 (s); OH: 2.45 (d, *J* = 8 Hz); isopropylidene: 1.53 (s) and 1.51 ppm (s). ^{13}C NMR: 207.45 (s); 137.65 (s); 136.93 (s); 130.83 (d); 126.08 (d); 113.86 (s); 96.38 (s); 89.69 (d); 75.78 (d); 74.87 (s) 44.19 (d); 38.67 (d); 31.65 (t); 28.73, 27.88, 27.04, 23.92 and 23.46 (2 d and 4 q); 23.01 (s); 18.00 (q); 15.40 (q); 15.27 ppm (q).

c) Acetylation of 20-deoxyingenol (**13**) affording the 3,5-diacetate (**14**): R_f (ether/petroleum ether 1/1): 0.55. MS: *m/e* = 416 (M^+). IR (KBr): 3490 (OH); 1738, 1713 cm^{-1} (CO); UV: λ (ε) = 193 nm (14160); λ_{max} (ε) = 290 nm (210); ^{1}H NMR: 1-H: 6.11 (d, *J* = 1.5 Hz); 7-H: 5.89 (m); 5-H: 5.23 (s, br.); 3-H: 4.95 (s); 8-H: 4.2 (m); 19-H_3: 1.78 (s. br.); 20-H_3: 1.58 (s. br.); 16-H_3, 17-H_3: 1.11 (s) and 1.07 (s); 18-H_3: 1.00 (d, *J* = 7.5 Hz); 14-H: 0.88 (superimposed); OH: 3.23 (s); 2 acetates: 2.28 (s); 2.16 ppm (s). CD (MeOH): λ ($\Delta\varepsilon$) = 302 (+0.475), 270 (−0.704), 242 (+0.290), 224 nm (−2.43).

d) Benzoylation of 20-deoxyingenol (**13**) affording the 3,5-dibenzoate (**24**): Benzoyla-

tion was carried out like the acetylation with benzoyl chloride instead of acetic anhydride. The spectral data of **24** are identical with those of compound P_8 (see below).

Euphorbia factor H_4 (18)

R_f (ether/petroleum ether 1/1): 0.29. MS: *m/e* = 414 (M^+), 314, 296. IR (CH_2Cl_2): 3590, 3490 (OH); 1715 (CO); 1640 cm^{-1} (C=C). UV: λ (ε) = 197 nm (15520); λ_{max} (ε) = 214 (13300); 294 (330), 328 nm (sh, 200). ^1H NMR: 1-H: 5.98 (m); 7-H: 5.89 (m); 5-H: 5.25 (s, br.); 8-H: 4.22 (m); 3-H: 3.79 (s); 19-H_3: 1.84 (s, br.); 20-H_3: 1.60 (s, br.); 16-H_3, 17-H_3: 1.15 (s) and 1.07 (s); 18-H_3: 0.98 (d, *J* = 7 Hz); 3.66 (s, imp.); OH: 3.91 (s); *ca.* 2.5 (superimposed); angelate: 1 olefinic H: 6.16 (m); 2 olefinic CH_3: 2.04–1.97 ppm (m).

a) Preparation of the acetonide **16** of factor H_4: R_f (petroleum ether/ether 3/2): 0.55. MS: *m/e* = 454 (M^+). IR (CH_2Cl_2): 1710 (CO) cm^{-1}. UV: λ (ε) = 194 nm (15820); λ_{max} (ε) = 215 (sh, 12120); 297 nm (410). ^1H NMR: 7-H: 5.85 (m); 1-H: 5.78 (m); 5-H: 5.34 (s); 3-H: 4.33 (s); 8-H: 4.1 (m); 19-H_3: 1.84 (d, *J* = 1.5 Hz); 20-H_3: 1.78 (s, br.); 16-H_3, 17-H_3: 1.16 (s) and 1.03 (s); angelate: 1 olefinic H: 6.03 (m); 2 olefinic CH_3: 2.04–1.97 (m); isopropylidene: 1.46 (s) and 1.40 ppm (s).

Euphorbia factor H_7 (20)

R_f (ether/petroleum ether 1/1): 0.33. MS: *m/e* = 480 (M^+), 314, 296. IR (CH_2Cl_2): 3590, 3490 (OH); 1715 (C=O); 1610 cm^{-1} (C=C). UV (MeOH): λ (ε) = 194 nm (14360); λ_{max} (ε) = 258 (sh, 5300); 270 (sh, 7680); 282 (sh, 11080); 309 nm (18170). ^1H NMR: *ca.* 8 olefinic H: 8.05–5.3 (m, including 1-H, 7-H); 5-H: 5.21 (s, br.); 8-H: 4.22 (m); 3-H: 3.79 (d, *J* = 4 Hz); 3.67 (s, imp.); 19-H_3: 1.85 (s, br.); 20-H_3: 1.62 (s, br.); 16-H_3, 17-H_3: 1.16 (s) and 1.07 (s, superimposed with m); OH: 3.91 ppm (d, *J* = 7 Hz).

a) Preparation of the acetonide **22** of factor H_7: R_f (petroleum ether/ether 3/2): 0.53. MS: *m/e* = 520. IR (CH_2Cl_2): 1715 (C=O); 1610 cm^{-1} (C=C). UV: λ (ε) = 197 nm (12990); λ_{max} (ε) = 258 (sh, 4050); 272 (sh, 5330); 282 (sh, 7000); 309 nm (11300). ^1H NMR: 6 olefinic H: 7.7–5.9 (m, superimposed); 1-H, 7-H: 5.8 (m, superimposed); 5-H: 5.33 (s, br.); 3-H: 4.31 (s, br.); 8-H: 4.05 (m); 3.66 (s, imp.); 19-H_3: 1.83 (s, br.); 20-H_3: 1.71 (s, br.); 16-H_3, 17-H_3: 1.19 (s) and 1.04 ppm (s).

b) Transesterification of factor H_7 affording 20-deoxyingenol (**13**) and unsaturated methyl esters: Spectral data of **13** see above. Catalytic hydrogenation of the unsaturated methyl esters (UV: λ = 302 nm) yielded methyl decanoate (rel. int. 100%) and others (< 5%) as identified by GLC analysis with authentic reference.

Euphorbia factor P_2 (19)

R_f (ether/petroleum ether 1/1): 0.31. MS: *m/e* = 436 (M^+), 314, 296. IR (CH_2Cl_2): 3590, 3490 (OH); 1715 (CO); 1598 cm^{-1} (C=C). UV: λ (ε) = 197 nm (34930); λ_{max} (ε) = 229 (14000); 267 (sh, 1070), 273 (1110), 280 (970), 300 nm (sh, 280). ^1H NMR: 1-H: 6.02 (d, *J* = 1.5 Hz); 7-H: 5.93 (m); 5-H: 5.44 (s, br.); 8-H: 4.3 (m); 3-H: 3.86 (s); 19-H_3: 1.84 (s, br.); 20-H_3: 1.62 (s, br.); 16-H_3, 17-H_3: 1.18 (s) and 1.08 (s); 18-H_3: 0.98 (d, *J* = 7 Hz); OH: 4.07 (s, br.); 2.8–2.2 (superimposed); benzoate: 8.3–7.3 ppm (m).

a) Preparation of the acetonide **21** of factor P_2: R_f (petroleum ether/ether 3/2): 0.52. MS: *m/e* = 476 (M^+). IR (CH_2Cl_2): 1715 (CO); 1598, 1580 cm^{-1} (C=C). UV: λ (ε) = 198 nm (43140); λ_{max} (ε) = 230 (14760), 274 (1050), 281 (880), 302 nm (sh, 210). ^1H NMR: 1-H: 5.91 (d, *J* = 1.5 Hz); 7-H: 5.88 (m); 5-H: 5.50 (s); 3-H: 4.38 (s); 8-H: 4.04 (m); 19-H_3: 1.86 (d, *J* = 1.5 Hz); 20-H_3: 1.74 (s, br.); 16-H_3, 17-H_3: 1.16 (s) and 1.04 (s); 18-H_3: 1.08 (d, *J* = 7 Hz); benzoate: 8.2–7.3 (m); isopropylidene: 1.50 (s) and 1.38 ppm (s).

Euphorbia factor P_7 (23)

R_f (ether/petroleum ether 1/1): 0.26. MS: *m/e* = 430 (M^+), 314, 296. IR (CH_2Cl_2): 3590, 3490 (OH); 1715 cm^{-1} (CO). UV: λ (ε) = 193 nm (15660); λ_{max} (ε) = 291 nm (280). ^1H NMR: 1-H 5.92 (d, *J* = 1.5 Hz); 7-H: 5.88 (m, superimposed with 1-H); 5-H: 5.20 (s, br.); 8-H: 4.22 (m); 3-H: 3.78 (d, *J* = 6 Hz, br.); 4 H: 2.6–2.1 (m); 19-H_3: 1.84 (d, *J* = 1.5 Hz); 20-H_3: 1.63 (s, br.); *ca.* 17 H: 1.2–0.9; OH: 3.68 (s); 2.23 ppm (d, *J* = 6 Hz).

Compound P_8 (24)

R_f (ether/petroleum ether 1/1): 0.54. MS: parent ion *m/e* = 540 (M^+). IR (CH_2Cl_2): 3560 (OH); 1718 (CO); 1598, 1581 cm^{-1} (C=C). UV: λ (ε) = 196 nm (81650); λ_{max} (ε) = 230 (28720); 266 (sh, 1870); 274 (2040); 281 (1690); 305 nm (sh, 210). ^1H NMR: 1-H: 6.27 (m); 7-H: 5.93 (m); 5-H: 5.63 (s); 3-H: 5.36 (s); 8-H: 4.33 (m); 19-H_3: 1.86 (d, *J* = 1.5 Hz); 20-H_3: 1.60 (s, br.); 16-H_3, 17-H_3: 1.11 (s) and 1.07 (s); OH: 3.66 (s); 2 benzoates: 8.4–7.3 ppm (m). ^{13}C NMR: 206.54 (s); 167.80 (s); 166.70 (s); 135.83 (s); 134.79 (s); 133.55 (s and d); 133.29 (d); 132.97 (d); 130.37 (2 d); 129.85 (2 d); 129.40 (s); 128.49 (2 d); 126.34 (2 d); 86.24 (s); 83.51 (d); 78.51 (d); 77.34 (imp.);

72.27 (s); 43.61 (d); 38.98 (d); 31.20 (t); 28.47 (q); 24.37 (s); 23.27 (d); 23.07 (d); 21.38 (q); 17.09 (q); 15.56 ppm (2 q).

Compound P_9 (**25**)

R_f (ether/petroleum ether 1/1): 0.61. MS: *m/e* = 534 (M^+). IR (CH_2Cl_2): 3550 (OH); 1710 (C=O); 1590, 1570 cm^{-1} (C=C). UV: λ (ε) = 195 nm (43540); λ_{max} (ε) = 230 (13890), 274 (1410); 282 (1250); 297 nm (sh, 500). ^1H NMR: 1-H: 6.11 (m); 7-H: 5.86 (m); 5-H: 5.49 (s, br.); 3-H: 5.04 (s); 8-H: 4.2 (m); 19-H_3: 1.77 (d, J = 1.5 Hz); 20-H_3: 1.56 (s); 16-H_3, 17-H_3: 1.13 (s) and 1.07 (s); 6 H: 1.1–0.8; OH: 3.44 (s, br.); benzoate: 8.2–7.3 ppm (m).

3. Derivatives of 20-deoxy-16-hydroxyingenol (**26**)

Euphorbia factor $\mathbf{Q_1}$ (**30**)

R_f (ether/petroleum ether = 4/1): 0.53. MS: *m/e* = 472 (M^+), 330, 312. IR (KBr): 3490 (OH); 1730, 1718 (CO); 1640 cm^{-1} (C=C). UV: λ (ε) = 196 nm (17880); λ_{max} (ε) = 211 (15930), 285 nm (280). ^1H NMR: 1-H: 6.02 (d, J = 1.5 Hz); 7-H: 5.70 (m); 3-H: 5.44 (s); 16-H_2: 4.16 ± 0.1 (J_{AB} = 12 Hz); 8-H: 4.12 (superimposed); 5-H: 3.67 (d, J = 7 Hz); 19-H_3, 20-H_3: 1.78 (m); 17-H_3: 1.1 (s); 14-H: 1.08 (superimposed); 18-H_3: 0.95 (d, J = 7 Hz); 13-H: 0.85 (superimposed); OH: 3.5 (s); 3.28 (d, J = 7 Hz); acetate: 2.06 (s); angelate: 1 olefinic H: 6.12 (m); 2 olefinic CH_3: 1.96–1.92 ppm (m).

a) Transesterification (reaction time: 5 h) of factor $\mathbf{Q_1}$ affording **26, 27** and **28**: In deviation from the general instructions 5×10^{-3} M $NaOCH_3$ in methanol was used for transesterification.

20-Deoxy-16-hydroxyingenol (**26**)

26 was characterized as its 3,5,16-triacetate **29,** spectral data see below.

20-Deoxy-16-hydroxyingenol-3-angelate (**27**)

MS: *m/e* = 430 (M^+). ^1H NMR: 1-H: 6.08 (d, J = 1.5 Hz); 7-H: 5.75 (m); 3-H: 5.45 (s); 8-H: 4.2 (m); 16-H_2: 3.85 ± 0.1 (J_{AB} = 12 Hz); 5-H: 3.65 (s); 19-H_3, 20-H_3: 1.8 (m); 17-H_3: 1.15 (s); 18-H_3: 0.95 (d, J= 7 Hz); 13-H, 14-H: 0.8; OH: 3.68, 3.12 ppm; angelate: 1 olefinic H: 6.17 (m); 2 olefinic CH_3: 2.0–1.96 ppm (m).

20-Deoxy-16-hydroxyingenol-5-angelate (**28**)

MS: *m/e* 430 (M^+). ^1H NMR: 1-H: 6.0 (d, J = 1.5 Hz); 7-H: 5.87 (m); 5-H: 5.25 (s); 8-H: 4.4 (m); 16-H_2: 3.82 (s); 3-H: 3.75 (s); 19-H_3: 1.85 (d, J = 1.5 Hz); 20-H_3: 1.56 (s, br.); 17-H_3: 1.15 (s); 18-H_3: 0.95 (d, J = 7 Hz); 13-H, 14-H: 0.85 (superimposed); OH: 3.96; 2.45; angelate: 6.15 (m); 2.01–1.97 ppm (m).

Euphorbia factor $\mathbf{P_6}$ (**33**)

R_f (ether/petroleum ether 3/1): 0.38. MS: *m/e* = 556 (M^+), 312, 294. IR (CH_2Cl_2): 3560 (OH, br.); 1715 (CO); 1600, 1580 cm^{-1} (C=C). UV: λ (ε) = 193 nm (80120); λ_{max} (ε) = 228 (29310), 266 (sh, 1420), 273 (1770), 280 (1380), 302 (sh, 210), 324 nm (sh, 80). ^1H NMR: 1-H: 6.16 (m); 7-H: 5.76 (m); 3-H: 5.73 (s); 16-H_2: 4.46 ± 0.11 (J_{AB} = 12 Hz); 8-H: *ca.* 4.22 (superimposed); 5-H: 3.77 (d, J = 7 Hz); 19-H_3: 1.84 (s, br.); 20-H_3: 1.76 (s, br.); 17-H_3: 1.22 (s); 18-H_3: 1.06 (d, J = 7 Hz); OH: 3.67 (s); 3.22 (d, J = 7 Hz); 2 benzoates: 8.2–7.3 ppm (m).

a) Transesterification (reaction time 1 h) of factor $\mathbf{P_6}$ affording 20-deoxy-16-hydroxyingenol-16-benzoate (**34**): R_f (dichloromethane/acetone 4/1): 0.41. MS: *m/e* = 452 (M^+). IR (CH_2Cl_2): 3460 (br., OH); 1710 (CO); 1596 cm^{-1} (C=C). UV: λ (ε) = 195 nm (42130); λ_{max} (ε) = 228 (12520), 267 (sh, 770), 273 (850), 280 (730), 297 nm (sh, 180). ^1H NMR: 1-H: 5.92 (d, J = 1.5 Hz); 7-H: 5.69 (m); 16-H_2: 4.51 (s); 3-H: 4.40 (s); 8-H: 4.2 (m); 5-H: 3.43 (s, br.); 19-H_3: 1.84 (d, J = 1.5 Hz); 20-H_3: 1.72 (s, br.); 17-H_3: 1.26 (s); 18-H_3: 0.97 (d, J = 7 Hz); OH: 4.2 (br.) and 3.2–2.5 ppm (superimposed); benzoate: 8.1–7.25 ppm (m).

b) Preparation of the acetonide **35** from **34:** R_f (ether/petroleum ether 3/1): 0.57. MS: *m/e* = 492 (M^+). IR (CH_2Cl_2): 3560 (OH); 1710 (CO); 1600 cm^{-1} (C=C). UV: λ (ε) = 194 nm (43700); λ_{max} (ε) = 228 (14670), 267 (sh, 770), 273 (920), 281 (810), 298 nm (sh, 200). ^1H NMR: 1-H, 7-H: 5.84 (m, superimposed); 3-H: 4.69 (s); 16-H_2: 4.56 ± 0.04 (J_{AB} = 12 Hz); 8-H: 4.22 (m); 5-H: 3.77 (d, br., J = 8 Hz); 19-H_3: 1.87 (s); 20-H_3: 1.79 (s, br.); 17-H_3: 1.22 (s); 18-H_3: 0.98 (d, J = 7 Hz); OH: 2.50 (d, J = 8 Hz); benzoate: 8.2–7.3 (m); isopropylidene: 1.53 (s) and 1.47 ppm (s).

c) Transesterification of **35** affording 20-deoxy-16-hydroxyingenol-3,4-acetonide (**36**): R_f (dichloromethane/acetone 4/1): 0.36. MS: *m/e* = 388 (M^+). IR (CH_2Cl_2): 3610, 3560 (br., OH); 1717 cm^{-1} (CO). UV: λ (ε) = 194 nm (11510); λ_{max} (ε) = 290 nm (220). ^1H NMR: 7-H: 5.88 (m); 1-H: 5.83 (m); 3-H: 4.69 (s); 8-H: 4.2 (m); 16-H_2, 5-H: 3.88 (superimposed); 19-H_3, 20-H_3: 1.87 (m, superimposed); 17-H_3: 1.17 (s); 18-H_3: 1.00 (d, J = 7 Hz); OH: 2.8–2.5 (br.); isopropylidene: 1.56 (s) and 1.51 ppm (s).

d) Transesterification (reaction time 24 h) of factor **P_6** affording 20-deoxy-16-hydroxyingenol (**26**): R_f (ethyl acetate): 0.20, staining: dark-brown. MS: *m/e* = 348 (M^+). IR (KBr): 3600–3200 (br., OH); 1705 cm^{-1} (CO). UV: λ (ε) = 194 nm (11510); λ_{max} (ε) = 2900 nm (220). ^1H NMR: 1-H: 5.97 (m); 7-H: 5.80 (m); 3-H: 4.42 (s); 8-H: 4.22 (m); 16-H_2: 3.80 ± 0.05 (J_{AB} = 12 Hz); 5-H: 3.50 (s, br.); 19-H_3: 1.87 (s); 20-H_3: 1.80 (s); 18-H_3: 0.96 (d, J = 7 Hz); 4 OH: 3.4–2.1 ppm (br.). ^{13}C NMR (CD_3OD): 210.63 (s); 141.22 (s); 140.70 (s); 129.79 (d); 123.74 (s); 86.18 (s); 81.24 (d); 77.27 (d); 73.96 (s); 63.30 (t); 44.52 (d); 40.81 (d); 31.65 (t); 31.13 (s); 24.96, 24.76, 24.57 (2 d and 1 q); 22.49 (q); 17.15 (q); 15.60 ppm (q).

e) Acetylation of 20-deoxy-16-hydroxyingenol (**26**) affording 20-deoxy-16-hydroxyingenol-3,5,16-triacetate (**29**): R_f (ether/petroleum ether 1/1): 0.18. MS: *m/e* = 474 (M^+). IR (CH_2Cl_2): 3550 (OH); 1730 cm^{-1} (CO). UV: λ (ε) = 194 nm (11760); λ_{max} (ε) = 283 nm (180). ^1H NMR: 1-H: 6.06 (d, J = 1.5 Hz); 7-H: 5.81 (m); 5-H: 5.20 (s, br.); 3-H: 4.91 (s); 8-H: 4.28 (m); 16-H_2: 4.20 ± 0.08 (J_{AB} = 12 Hz); 19-H_3: 1.77 (d, J = 1.5 Hz); 20-H_3: 1.56 (s); 17-H_3: 1.13 (s); 18-H_3: 0.98 (d, J = 7 Hz); OH: 3.20 (s); 3 acetates: 2.28 (s), 2.13 (s) and 2.09 ppm (s).

Compound P_1 (**32**)

R_f (ether/petroleum ether 3/1): 0.48. MS: *m/e* = 556 (M^+), 312, 294. IR (KBr): 3440 (br., OH); 1715 (CO); 1595, 1580 cm^{-1} (C=C), UV: λ (ε) = 195 nm (83220); λ_{max} (ε) = 229 (30140), 266 (sh, 1950), 273 (2160), 281 nm (1810). ^1H NMR: 1-H: 6.00 (d, J = 1.5 Hz); 7-H: 5.90 (m); 5-H: 5.46 (s, br.); 16-H_2: 4.55 (s, br.); 8-H: 4.5 (superimposed); 3-H: 3.87 (s); 19-H_3: 1.84 (s); 20-H_3: 1.55 (s); 17-H_3: 1.24 (s); 18-H_3: 0.99 (d, J = 7 Hz); OH: 4.20 (s); 3.3 (br.); 2 benzoates: 8.3–7.3 ppm (m). ^{13}C NMR: 206.93 (s); 167.15 (s); 166.44 (s); 139.66 (s); 135.70 (s); 133.62 (d); 130.11 (d); 129.66 (d); 129.33 (s); 129.20 (d); 128.62 (d); 128.49 (d); 124.85 (d); 85.07 (s); 79.87 (d); 77.34 (d); 72.98 (s); 66.48 (t); 43.61 (d); 39.45 (d); 30.87 (t); 27.82 (s); 24.44 (q); 24.05 (d); 23.85 (d); 21.45 (q); 17.03 (q); 15.47 ppm (q).

a) Preparation of the acetonide **31** of compound P_1: R_f (ether/petroleum ether 1/1): 0.4. MS: *m/e* = 596 (M^+). IR (KBr): 1715 (CO); 1595, 1580 cm^{-1} (C=C). UV: λ (ε) = 196 nm (76970); λ_{max} (ε) = 230 (28100), 267 (sh, 1750), 273 (1970), 281 nm (sh, 1650). ^1H NMR: 1-H: 5.93 (d, J = 1.5 Hz); 7-H: 5.91 (m, superimposed); 5-H: 5.52 (s); 16-H_2: 4.58 ± 0.16 (J_{AB} = 12 Hz); 3-H: 4.39 (s); 8-H: 4.23 (m); 19-H_3: 1.87 (d, J = 1.5 Hz); 20-H_3: 1.73 (s, br.); 17-H_3: 1.22 (s); 18-H_3: 1.06 (d, J = 7 Hz); 2 benzoates: 8.2–7.2 (m); isopropylidene: 1.47 (s) and 1.38 ppm (s).

4. Derivatives of 20-deoxy-13,16-dihydroxyingenol (**37**)

Euphorbia factor P_5 (**38**)

R_f (ether/petroleum ether 3/1): 0.37, staining: brown-grey. MS: *m/e* = 676 (M^+), 328, 310. IR (CH_2Cl_2): 3555, 3500 (br., OH); 1720 (CO); 1599, 1581 cm^{-1} (C=C). UV: λ (ε) = 193 nm (96260); λ_{max} (ε) = 196 (90190); 231 (37290), 266 (sh, 2510), 274 (2830), 281 nm (sh, 2370). ^1H NMR: 1-H: 6.18 (d, J = 1.5 Hz); 3-H: 5.77 (s); 7-H: 5.74 (m, superimposed); 16-H_2: 4.56 ± 0.11 (J_{AB} = 12 Hz); 8-H: 4.3 (superimposed); 5-H: 3.79 (s); 19-H_3: 1.82 (d, J = 1.5 Hz); 20-H_3: 1.72 (s, br.); 14-H: 1.61 (d, J = 12 Hz, partly superimposed); 17-H_3: 1.29 (s); 18-H_3: 0.99 (d, J = 7 Hz); OH: 3.7, 3.1–2.0 (superimposed); 3 benzoates: 8.25–7.35 ppm (m).

Compound P_3 (**39**)

R_f (ether/petroleum ether 3/1): 0.36. MS: *m/e* = 690 (M^+), 328, 310. UV: λ (ε) = 194.5 nm (70080); λ_{max} (ε) = 228 (21130), 268 (sh, 1500); 274 (1640), 281 nm (1390). ^1H NMR: 1-H: 6.10 (d, J = 1.5 Hz); 7-H, 3-H: 5.65 (m, superimposed); 16-H_2: 4.41 ± 0.10 (J_{AB} = 12 Hz, superimposed with 8-H and imp.); 5-H: 3.73 (s, br.); 19-H_3: 1.84 (d, J = 1.5 Hz); 20-H_3: 1.73 (s, br.); 14-H: 1.50 (partly superimposed); 17-H_3: 1.14 (s); OH: *ca.* 1.8 (superimposed); phenylacetate: arom. H: 7.28 (s, partly superimposed); methylene group: 3.57 (s); 2 benzoates: 8.2–7.2 ppm (m).

Compound P_4 (**40**)

R_f (ether/petroleum ether 3/1): 0.36. MS: *m/e* = 614 (M^+), 328, 310. IR (CH_2Cl_2): 3680–3300 (br., OH); 1712 (CO); 1597, 1580 cm^{-1} (C=C). UV: λ (ε) = 195 nm (62900); λ_{max} (ε) = 229 (22570), 266 (sh, 1860), 273 (2150), 280 nm (1940). ^1H NMR: 1-H: 6.12 (d, J = 1.5 Hz); 3-H, 7-H: 5.65 (m, superimposed); 16-H_2: 4.43 ± 0.10 (J_{AB} = 12 Hz); 8-H: 4.25 (m); 5-H: 3.74 (s, br.); 19-H_3: 1.85 (d, J = 1.5 Hz); 20-H_3: 1.73 (s, br.); 14-H: 1.45 (d, J = 13 Hz); 17-H_3: 1.26 (s); OH: 3.6 and 3.0–2.0 (superimposed); acetate: 2.03 (s); 2 benzoates: 8.2–7.3 ppm (m).

a) Isomerisation of compound P_4 affording **41:** After storage of compound P_4 (**40**) for six months in ethyl acetate by tlc an additional compound was detected and isolated by prep. TLC to yield 30% of 20-deoxy-13,16-dihydroxyingenol-13-

acetate-5,16-dibenzoate (**41**). R_f (ether/petroleum ether 3/1): 0.37. MS: m/e = 614 (M^+). ^{1}H NMR: 1-H: 5.98 (d, J = 1.5 Hz); 7-H: 5.81 (m); 5-H: 5.40 (s, br.); 16-H_2: 4.55 (s); 8-H: 4.4 (m); imp.: 4.22 and 4.17; 3-H: 3.83 (s); 19-H_3: 1.84 (d, J = 1.5 Hz); 20-H_3: 1.55 (s, br.); 17-H_3: 1.39 (s); imp.: 1.35 (s); 18-H_3:1.02 (d, J = 7 Hz); OH: 3.9 and 1.6; acetate: 2.03 (s); 2 benzoates: 8.2–7.3 ppm (m).

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