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IRRITANT PRINCIPLES OF THE SPURGE FAMILY (EUPHORBIACEAE) XIII.[1] OLIGOCYCLIC AND MACROCYCLIC DITERPENE ESTERS FROM LATICES OF SOME *EUPHORBIA* SPECIES UTILIZED AS SOURCE PLANTS OF HONEY[2]

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ABSTRACT.—The latices of the three South African species *Euphorbia ledienii, Euphorbia coerulescens,* and *Euphorbia triangularis*, belonging to a group of cactiform Euphorbias locally called "Noors," were shown to contain four 12-mono- and three 13,20-diesters of the tetracyclic tigliane type parent alcohol 12-deoxyphorbol [**1**]. In addition, two 13,16-di- and two 13,16,20-triesters of the related 12-deoxy-16-hydroxyphorbol [**9**] were obtained. Ester groups in **1** and **9** are made up of acetic, isobutyric, tiglic, angelic, and 2-methylbutyric acid; their positions in the parent alcohols were identified. The free parent alcohols were not detected in the latices. On the mouse ear all esters isolated showed moderate irritant activity as compared to the tigliane-type standard TPA. In addition to the oligocyclics listed above, the latex of *E. ledienii* also yielded five esters of the macrocylic, lathyrane type parent alcohol ingol [**14**]. In the two triesters and three tetraesters the ester groups were made up of acetic, tiglic, and 2-methylbutyric acid (positions in **14** unidentified). None of the ingol esters showed irritant activity.

In arid zones of South Africa several thorny, cactiform *Euphorbia* species grow abundantly and are called collectively "Noors doring" or "Noors" (2). They cover considerable areas almost like monocultures (stands), e.g., in the semidesert Karoo, the eastern region of the Cape Province. Some are utilized for a variety of agricultural purposes (2). Also, it is well known to local beekeepers that "stands" of noors, such as *Euphorbia ledienii* Berg., *Euphorbia coerulescens* Haw., *Euphorbia triangularis* Desf., *Euphorbia ingens* E. Mey, *Euphorbia tetragona* Haw., and *Euphorbia cooperi* N.E.Br., are highly attractive to honey bees, and they use them as an inexpensive means to feed bees or to harvest the honey (Noors honey) (3–5). In the literature it is reported that such honey causes a strong burning sensation in the mouth and throat, often persisting for some hours; it is reported to increase even on drinking water (3–6). At times, this honey creates a problem to agriculturists for the quantity of Noors honey annually rendered unsaleable or greatly reduced in value may be reckoned as several tons (4). Thus, an early investigation of Noors honey, using its peculiar taste as a lead to concentrate and identify the acrid principles, was aimed at developing means of their elimination (4).

In the meantime it has become known that certain polyfunctional diterpene esters (DTE) of the tigliane, ingenane, and daphnane type are responsible for the acrid properties of *Euphorbia* species; these esters occur in the latices of the plants and hence probably in all of their parts. Recently it was demonstrated for a variety of *Euphorbia* species including some of the Noors species that laticifers may approach and invade even their nectar glands (nectaries) (7,8). In mouse skin many of the irritant DTE from *Euphorbia* latices are cocarcinogens of the tumor-promoter type (14). If taken internally, they must be considered risk factors of cancer (5–17).

As a background for an analytical survey of honeys collected from major Noors

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[1]For Communication XII see Fürstenberger and Hecker (1); beginning with Part XIII the topic of the series was slightly modified.

[2]Dedicated to Prof. Dr. H. Schildknecht, Institute of Organic Chemistry, University of Heidelberg, on the occasion of his 65th birthday, in appreciation of his many fascinating contributions to natural product chemistry.

[3]The investigations on latex of *Euphorbia ledienii* and *Euphorbia triangularis* are part of the Dissertation, University of Heidelberg, 1984.

species (18–20) and other plants of the Euphorbiaceae, the latices of *E. ledienii*, *E. coerulescens*, and *E. triangularis* were carefully investigated (or reinvestigated) to develop for their diterpene contents appropriate systematic and quantitative analytical separation procedures and to provide individual diterpenes as references for investigations of Noors honey.

RESULTS

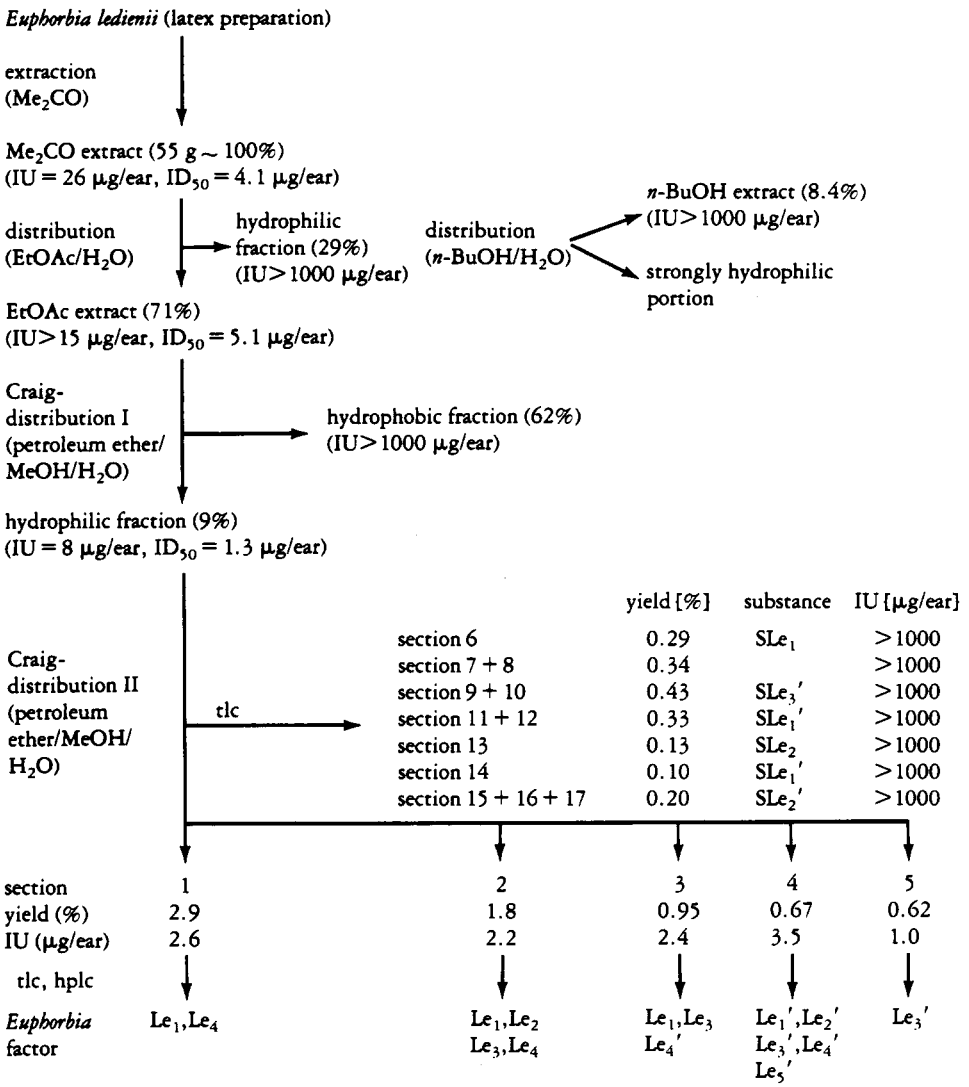
SYSTEMATIC QUANTITATIVE SEPARATION PROCEDURES YIELDING DITERPENOID *EUPHORBIA* FACTORS OR COMPOUNDS AND THEIR QUANTITATIVE BALANCE.—The Me_2CO extracts of the latices of all three species exhibited considerable irritant activity on the mouse ear. They were enriched with respect to irritant activity in a systematic fractionation procedure employing simple and multiple liquid-liquid distributions. The "sections" resulting from the latter were separated by tlc or hplc. Special care was taken to carry through the entire systematic fractionation procedures without substantial loss of irritant activity and to pinpoint virtually all oligocyclic and macrocyclic diterpenes present in the corresponding extracts of the latices. A typical example for a quantitative, analytical separation procedure, that for latex of *E. ledienii*, is given in Scheme 1.

To assign in a concise manner irritant diterpenoids purified from plants of the Euphorbiaceae (and Thymelaeaceae) yielding single spots in tlc (or single peaks in hplc), the term "factor" is used in conjunction with the systematic botanical name of the species [taxonomic system by Engler (21)]. As a rule, the genus of the species and the first letter of the specifying adjective is used (capital letter), e.g., *Euphorbia* factor C (*E. cooperi*) (9,10). If, by previous assignments, the first letter of the specifying adjective is occupied, its first two letters may be used, e.g., *Euphorbia* factor Le (*E. ledienii*). Multiplicity of factors in any one plant species is accounted for by indices starting with 1 and in arbitrary sequence, for example, "*Euphorbia* factors Le_1–Le_5". Tlc- or hplc-uniform diterpenoids exhibiting relatively weak or no irritant activity, which yield (e.g., by mild alkali catalyzed transesterification) more active compounds, are assigned by analogy as factors also (see above), but with prime, e.g., "*Euphorbia* factor Le′". This assignment was chosen to indicate the close toxicologic and chemical relationship of any such "cryptic factors" or "cryptic analogues", e.g., Euphorbia factor Le′, to the corresponding highly active factor *Euphorbia* factor Le. In contrast to "factors" a tlc- or hplc-uniform nonirritant diterpenoid which cannot be activated as above is termed "substance" in conjunction with the systematic botanical name of the species as above, using in addition capital S as a prefix, e.g., for *E. ledienii* "*Euphorbia* substance SLe". Multiplicity of the "substances" is denoted as for "factors" above. Any "substance" which loses an ester group, e.g., by mild alkali catalyzed transesterification, is assigned by analogy with prime, e.g., "*Euphorbia* substance SLe′".

This system of preliminary terminology and coding of "factors" and "substances" proved useful in isolation and in toxicologic characterization as well as in the general biological context of the polyfunctional diterpenes under consideration, especially as a means of differentiating in a simple manner the bio-active and bio-inactive diterpenoids.

LATEX OF *E. LEDIENII*.—From the systematic quantitative fractionation procedure (Scheme 1) *Euphorbia* factors Le_1–Le_4 and Le_1'–Le_5' were obtained together with *Euphorbia* substances SLe_1, SLe_2, SLe_1', SLe_2', and SLe_3'. Some of the characteristics of the uniform entities, including yields and irritant activities in terms of ID_{50}^{24} and I^{24}, are summarized in Table 1.

According to nmr and mass spectral data, the *Euphorbia* factors Le_1–Le_3 are the 13-



SCHEME 1. Flowsheet of the Systematic Quantitative Fractionation Procedure for the Latex Preparation of *Euphorbia ledienii* Monitored for Irritant Activities (IU) in Main- and Side-stream Fractions.

monoesters **2**, **3**, and **4** of 12-deoxyphorbol [**1**] with short chain aliphatic acids (Table 2, Figure 1). These esters were obtained previously from *E. triangularis* (11). *Euphorbia* factors Le_1'–Le_3' showed identical nmr spectra as the 20-acetates of 12-deoxyphorbol-13-monoesters obtained previously by partial syntheses (11). Thus, Le_1'–Le_3' represent the corresponding 20-acetates **6–8** of factors Le_1–Le_3 (**2–4**). In addition, in case of **6** the position of the acetyl residue was proven by partial transesterification yielding the 13-isobutyrate **2**.

Le_4 is identical with the previously described *Euphorbia* factor C, the 13-angelate-16-isobutyrate [**10**] of 12-deoxy-16-hydroxyphorbol [**9**]. In C the structure of the parent alcohol **9** (especially as a 16-hydroxy derivative) as well as the positions of the acyl residues were established previously (9, 10, 22, 23). Le_4' represents the 20-acetate **12** of Le_4 (**10**, Table 2, Figure 1), as proven by partial transesterification.

For Le_5', a 13,16,20-angelate, 2-methylbutyrate, acetate, no corresponding

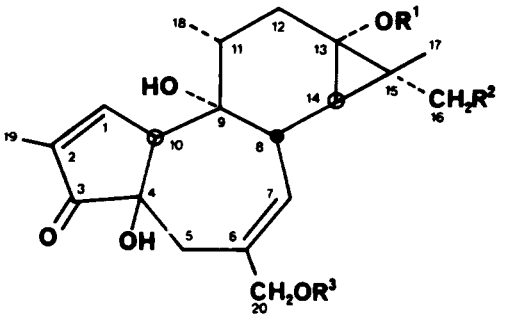
TABLE 1. Some Characteristics of *Euphorbia* Factors and Substances Isolated from the Latices of *Euphorbia ledienii*, *Euphorbia coerulescens*, and *Euphorbia triangularis* Including Irritant Activities on the Mouse Ear.

Factor/compound	Yield (%)[a]			R_f value[b]	ID_{50}^{24} (nmol/ear)	I^{24} ($nmol^{-1}$)	Structure
	E. ledienii	*E. coerulescens*	*E. triangularis*				
Esters of 12-deoxyphorbol [**1**]							
Le_1	0.17	0[c]	0.006	0.10	0.53	1.9	**2**
Le_2	0.075	0	0.025	0.10	0.28	3.6	**3**
Le_3	0.54	0.06	0	0.11	0.83	1.2	**4**
T_4	0	0	0.003	0.10	IU>7	—	**5**
Le_1'	0.022	0	0.066	0.44	1.5	0.7	**6**
Le_2'	0.027	0	0.19	0.46	1.2	0.8	**7**
Le_3'	0.14	0.74	0.15	0.47	1.5	0.7	**8**
Esters of 12-deoxy-16-hydroxyphorbol [**9**]							
Le_4	0.093	0	0.03	0.10[d]	0.25	4.0	**10**
Coe_2	0	0.07	0	0.10[d]	n.d.[f]	—	**11**
Le_4'	0.16	0	0.16	0.38[d]	0.91	1.1	**12**
Le_5'	0.067	0.25	0	0.41[d]	2.4	0.4	**13**
Esters of ingol [**14**]					IU (μg/ear)		
SLe_1	0.006	0	0	0.20[e]	>1000	—	**15**
SLe_2	0.015	0	0	0.40[e]	>1000	—	**16**
SLe_1'	0.13	0	0	0.45[e]	>1000	—	**17**
SLe_2'	0.037	0	0	0.48[e]	>1000	—	**18**
SLe_3'	0.086	0	0	0.30[e]	>1000	—	**19**

[a]Me_2CO extracts = 100%.
[b]Si gel, Et_2O-petroleum ether (4:1), brown spots after spraying with vanillin/H_2SO_4 and heating (100°).
[c]An entry of 0 indicates that no factor/substance was obtained.
[d]Spots staining blue-gray.
[e]Et_2O-petroleum ether (1:1), spots staining brown-gray.
[f]n.d. = not determined.

TABLE 2. Overview of the Irritant, Tigliane Type DTE as Obtained in This Paper and Identified Previously.

Parent alcohol	DTE structure	Ester moities	Species	Location	Previous reports
12-deoxyphorbol [**1**] . .	**5**	13-acetate	*Euphorbia triangularis*, (*Pimelea prostrata*)	New Zealand, Australia, South Africa	(20, 42–44)
	2	13-isobutyrate	*Euphorbia ledienii*, *E. triangularis*	South Africa	(9–11, 20)
	3	13-tiglate	*E. ledienii*, *E. triangularis*	South Africa	
	4	13-(2-methylbutyrate)	*E. ledienii*, *Euphorbia coerulescens*	South Africa	
	6	13-isobutyrate-20-acetate	*E. ledienii*, *E. triangularis*	South Africa	
	7	13-tiglate-20-acetate	*E. ledienii*, *E. triangularis*	South Africa	
	8	13-(2-methylbutyrate)-13-acetate	*E. ledienii*, *E. coerulescens*, *E. triangularis*	South Africa Botanical Garden, Kew	(20, 30)
12-deoxy-16-hydroxyphorbol [**9**] . .	**10**	13-angelate-16-isobutyrate	*E. ledienii*, *E. triangularis* *Euphorbia cooperi*	South Africa	(9–11, 20)
	11	13-angelate-16-(2-methylbutyrate)	*E. coerulescens*	South Africa	
	12	13-angelate-16-isobutyrate-20-acetate	*E. ledienii*, *E. triangularis* *E. cooperii*	South Africa	
	13	13-angelate-16-(2-methylbutyrate)-20-acetate	*E. ledienii*, *E. coerulescens*	South Africa	



1 $R^1=R^2=R^3=H$
2 R^1=isobutyl; $R^2=R^3=H$
3 R^1=tigloyl; $R^2=R^3=H$
4 R^1=2-methylbutyryl; $R^2=R^3=H$
5 R^1=acetyl; $R^2=R^3=H$
6 R^1=isobutyryl; $R^2=H$; R^3=acetyl
7 R^1=tigloyl; $R^2=H$; R^3=acetyl
8 R^1=2-methylbutyryl; $R^2=H$; R^3=acetyl
9 $R^1=H$; R^2=OH; $R^3=H$
10 R^1=angeloyl; R^2=isobutyrate; $R^3=H$
11 R^1=angeloyl; R^2=2-methylbutyrate; $R^3=H$
12 R^1=angeloyl; R^2=isobutyrate; R^3=acetyl
13 R^1=angeloyl; R^2=2-methylbutyrate; R^3=acetyl

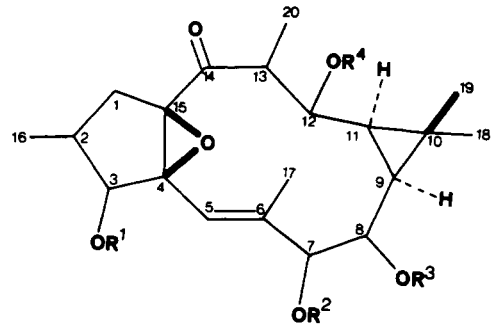
FIGURE 1. Chemical structures of the esters of 12-deoxyphorbol [**1**] and 12-deoxy-16-hydroxyphorbol [**9**] isolated from *Euphorbia ledienii*, *Euphorbia coerulescens*, and *Euphorbia triangularis*.

13,16-diester could be isolated. However, from *E. coerulescens* the 13-angelate-16-(2-methylbutyrate) of 12-deoxy-16-hydroxyphorbol (Coe_2) was obtained. By comparing the nmr spectra of Le_5' and Coe_2, the structure of Le_5' could be deduced to be 12-deoxy-16-hydroxyphorbol-13-angelate-16-(2-methylbutyrate)-20-acetate. The position of the acetate moiety was deduced by analogy to Le_4'.

In comparison to the standard irritant 12-*O*-tetradecanoylphorbol-13-acetate [TPA, $ID_{50}^{24}=0.016$ nmol/ear (24)] all *Euphorbia* factors Le are moderate irritants (ID_{50}^{24} values >0.25 nmol/ear, Table 1). The 13-monesters **2–4** of 12-deoxyphorbol [**1**] and also the 13,16-diester **10** of 12-deoxy-16-hydroxyphorbol [**9**] show similar irritant activities as the corresponding 20-acetates **6–8**, **12**, and **13**, respectively. Within the variability of the biological assay a tendency of higher ID_{50}^{24} in the 20-acetates is apparent (see "cryptic irritants" in Discussion).

The nmr and mass spectral data of the substances SLe_1, SLe_2, SLe_1', SLe_2', and SLe_3' show that they all are esters of ingol [**14**], (Figure 2)[4]; SLe_1 and SLe_2 represent triesters (**15**,**16**) and SLe_1'–SLe_3' tetraesters (**17–19**). The number and nature of the short chain acyl residues were determined (Table 1, Figure 2), whereas their position in the diterpene moiety remained open. By an IU >1000 µg all of them are nearly inactive as irritants. The parent alcohols **1**, **9**, and **14** of the isolated DTE were not obtained in the quantitative analytical separation procedure (Scheme 1).

[4]In contrast to previous notifications (25,26), it appears appropriate to assign absolute configurations in **14** only to the asymmetric centers in the five- and three-membered rings [by α or β relating 9α-H in **14** with H-14α in phorbol as the reference center (24)]. The chirality of the asymmetric centers, including those in the macrocyclic ring, reads 2*S*; 3*S*; 4*R*; 7*R*; 8*S*; 9*S*; 11*R*, 12*R*; 13*R*; 15*R*.



14 $R^1=R^2=R^3=R^4=H$
15 $R^1=H$; R^2,R^3,R^4=acetyl, tigloyl, tigloyl
16 R^1,R^2,R^3=acetyl, 2-methylbutyryl, tigloyl, $R^4=H$
17 R^1,R^2,R^3,R^4=acetyl, acetyl, tigloyl, tigloyl
18 R^1,R^2,R^3,R^4=acetyl, acetyl, 2-methylbutyryl, tigloyl
19 R^1,R^2,R^3,R^4=acetyl, acetyl, acetyl, tigloyl

FIGURE 2. Chemical structures of the esters of ingol [**14**] isolated from *Euphorbia ledienii*. Numbering system of the C-atoms in ingol given as revised by Lotter *et al.* (25) and Adolf and Hecker (26).

LATEX OF *E. COERULESCENS*.—The structures of three of the four *Euphorbia* factors isolated turned out to be identical with *Euphorbia* factors Le_3 [**4**], Le_3' [**8**], and Le_5' [**13**] as obtained from *E. ledienii*, carrying 12-deoxyphorbol or 12-deoxy-16-hydroxyphorbol as the parent alcohol. The fourth *Euphorbia* factor was identified as Coe_2, the structure of which was assigned by comparison to *Euphorbia* factor Le_4 to be 12-deoxy-16-hydroxyphorbol-13-angelate-16-(2-methylbutyrate) [**11**]. It is the 13,16-diester corresponding to Le_5', the 13-angelate-16-(2-methylbutyrate)-20-acetate **13** from the latex of *E. ledienii*. Coe_2, the 13,16-diester **11** was not present in *E. ledienii*, and its irritant activity was not determined. No ingol esters were present in latex of *E. coerulescens*. Also, the parent alcohols **1** and **9** of the isolated DTE were not obtained in the quantitative analytical separation procedures (Table 1).

LATEX OF *E. TRIANGULARIS*.—Eight *Euphorbia* factors were isolated from this latex (Tables 1,2). Seven of these are identical with *Euphorbia* factors Le: Le_1 [**2**], Le_2 [**3**], Le_1' [**6**], Le_2' [**7**], Le_3' [**8**], Le_4 [**10**], and Le_4' [**12**]. The eighth, *Euphorbia* factor T_4, was identified as 12-deoxyphorbol-13-acetate [**5**], not previously obtained from *E. triangularis* (11). It was described as a toxin of some Thymelaeaceae species (see Discussion) and is of little irritant activity (Table 1). No ingol esters were detected in the latex of *E. triangularis*. Also, the parent alcohols **1** and **9** of the isolated DTE were not obtained in the quantitative analytical separation procedure (Scheme 1).

DISCUSSION

Reports in the literature on the utilization of the three Noors species *E. ledienii*, *E. coerulescens*, and *E. triangularis* are contradictory in various regards. Also they are incomplete with regard to quality and quantity of irritant and tumor-promoting diterpenes contained in their latices. The regional geographic origin in South Africa of the plant sources used for previous investigations (see Table 2) was different from those of the three species investigated here.

E. ledienii, commonly known as "Suurnoors," is a spiny, leafless, bushy plant up to 2 m high with erected 4- to 7-angled branches. It grows in arid regions, frequently in the east part of the Cape Province in South Africa, the semidesert Karoo (2). The whole plant and the latex are said to be virulently poisonous (2,27). The latex of the plant was reported to contain esters of 12-deoxyphorbol and 12-deoxy-16-hydroxyphorbol; individual molecular entities were not identified (28,29).

E. coerulescens, also known as blue euphorbia or "Soetnoors," is a leafless bush with short spines up to 1.5 m high, with the branches more or less clustered or whorled and slightly constricted at intervals into elongated and oblong 4- to 5-angled segments (2). This species is indigenous to the same regions as *E. ledienii*. It is said that *E. coerulescens* is an important animal fodder in the karoo (2). Also, the latex of *E. coerulescens* is reported to be a weak irritant (30). Chemical examination of the latex revealed that mono- and diesters of 12-deoxyphorbol were present (30–34).

E. triangularis is a spiny succulent tree with a cylindric trunk growing up to a height of 18 m. It has various common names: "river euphorbia" or "Riviernaboom," indicating that it grows on riversides, especially in the Karoo, or "chandelier plant" referring to the candelabra-like arrangement of the triangled branches. "Naboom" is another indigenous name meaning "powerful" or "energetic," in reference to the properties of the latex (2,35). The milky latex is said to be non-irritant and reported to be utilized for the manufacture of inferior rubber, as a basis of chewing gum as well as for the confectionery trade (11). Chemical investigations of the latex of *E. triangularis* have shown so far the presence of various irritant diterpene esters of 12-deoxyphorbol (9–11, 28, 29, 34).

The analyses of the latices of *E. ledienii*, *E. coerulescens*, and *E. triangularis* presented here showed that all of them contain diterpenes of moderate skin irritant activity. They are of the tetracyclic tigliane type, i.e., esters of either 12-deoxyphorbol [**1**] or of 12-deoxy-16-hydroxyphorbol [**9**].

Some of the diterpene esters isolated from the latices are identical with those obtained previously from the same or other *Euphorbia* species [e.g., *E. cooperi* (9,10)] or else from a *Pimelea* species (Thymelaeaceae, Table 2). On the other hand the 12-deoxy-16-hydroxyphorbolesters **11** and **13** have not been described as yet. In the "cryptic irritants" identified, i.e., 20-acetates of the irritant 13-monoesters of 12-deoxy- and 16-hydroxyphorbolesters, the comparatively small difference between their ID_{50}'s is in some contrast to findings in long chain 13-mono-(or 12,13-) diesters of, for instance, 12-deoxyphorbol or phorbol and corresponding "cryptic irritants." As a rule, the former usually are considerably more active as corresponding 20-acetates and even more so as corresponding long chain 20-esters (15,19).

The latex of *E. ledienii* alone contained diterpene esters of the macrocyclic lathyrane type, i.e., of the polyfunctional diterpene ingol [**14**] (Table 1). Only **19** was isolated previously from various *Euphorbia* species (13, 36–39). The ingol esters **15–18** proved to be non-irritant on the mouse (IU>1000 μg/ear, Table 1), as expected from previous experience (13).

Species of Euphorbiaceae may be classified chemotaxonomically with regard to the principal structural types of diterpenes they contain (i.e., tigliane, ingenane, daphnane, lathyrane, and jatrophane types). To be meaningful such classification requires, of course, systematic and quantitative measures to account for all the diterpene types possibly involved. Thus, according to the data presented *E ledienii* may be termed a "tigliane/lathyrane species." *E. coerulescens* and *E. triangularis* correspondingly are "tigliane species." In addition to such a *qualitative classification* the balance of the systematic quantitative separation procedures of the latex preparations of all three species (e.g., Scheme 1) also reveal an important *quantitative* aspect: the ratio of biologi-

cally active DTE, e.g., of the tigliane type over the corresponding inactive tigliane parent alcohol(s) contained in any one of the latex preparations may be estimated to be aET/iaTP > 100 in account of the sensitivity of the detection methods used; aET = minimum amount of active esters of the tigliane type, iaTP = minimum amount of inactive tigliane-type parent alcohol(s). In analogy, for the corresponding lathyrane types iaEL/iaLP > 100 is valid: iaEL = minimum amount of inactive esters of the lathyrane type, iaLP = minimum amount of inactive lathyrane type parent alcohol(s). In other words, the three latex preparations investigated contain practically no free diterpene preparent alcohols which correspond to the DTE identified. Such ratio may be of toxicologic relevance as regards the honeys collected from the species investigated (19).

The diterpene esters of the tigliane type, besides being strong irritants, often are also potent tumor promoters of mouse skin (19). Such activity was demonstrated for the latices derived from *E. triangularis* and from another "Noors" species, *E. cooperi* (9–11). Because of these bioactivities, especially of the DTE contained in the latices of the species investigated, it is surprising that cattle would accept *E. coerulescens* as a fodder plant (2) or that the latex of *E. triangularis* was used as an inferior source of rubber for manufacturing chewing gum. Moreover, *E. ledienii* and *E. coerulescens* are registered (3), and *E. triangularis* is reported (27) to be used as a honey plant in South Africa. In this context Anderson *et al.* (3) note: "Some honeys straight from the comb have unacceptable characteristics. The best known South African example of this is the honey known locally as Noors honey. It is derived from some *Euphorbia* species, especially *E. ledienii* (Suurnoors). The honey contains a bitter principle that leaves a bitter, burning sensation in the mouth and throat. It is not immediately noticeable, appearing as an aftertaste, which may persist for an hour or more." Based upon this and similar previous observations (4), the working hypothesis was put forward that diterpenes occurring in the latices of Euphorbiaceae species may be secreted by the nectaries of the plants together with the nectar (18–20). This may be a problem of particular relevance to all those regions of the world where Euphorbiaceae occur in growths covering large areas, be it as weeds or as plantations of economic interest.

EXPERIMENTAL

PLANT MATERIAL.—The latices were collected in the standardized manner under MeOH for preservation (9,10). Collection was carried out by Mr. P. and Mrs. C. Hässler, Skeerpoort, in stands located in several parts of the Karoo, South Africa: *E. ledienii* near Kirkwood, *E. triangularis* near Jansenville, and *E. coerulescens* in the countryside near Jansenville. The plants were identified by material sent to and deposited at the National Herbarium, Botanical Research Institute, Department of Agriculture and Fisheries, Pretoria, South Africa.

ANALYTICAL METHODS.—Apparatus and methods of countercurrent distributions have been described previously (24,40,41). MN-Kieselgel P/UV_{254} from Macherey/Nagel & Co., Düren, FRG, was used for preparative tlc as well as precoated Merck tlc plates, Si gel $60F_{254}$. Spots were located at first under uv light (254 nm) and subsequently by spraying with vanillin/H_2SO_4 and heating to 100°. For analytical and preparative hplc, instruments of Water Associates, Milford, Massachusetts and Du Pont Company, Wilmington, Delaware were used employing uv detectors ($\lambda = 254$ nm).

SPECTRA.—Ir spectra were measured on a Perkin-Elmer spectral photometer 521 and uv spectra on a Beckman spectral photometer Acta M VI. Mass spectra were obtained with a Varian mass spectrometer MAT 711. ^{1}H-nmr spectra were done on a Bruker HX-90 spectrometer, in $CDCl_3$ with TMS ($\delta = 0.00$ ppm) as internal standard.

BIOLOGICAL ASSAYS.—Skin irritant activities of fractions, *Euphorbia* factors, and *Euphorbia* substances were determined in the standard assay on the ears of mice (24): the irritation unit (IU^{24}), read 24 h after administration, was determined on SIM mice. For comparison of relative irritant activities of fractions, the IU^{24} were used in the fractionation procedures throughout (Scheme 1). In the case of pure factors and substances, determination of the IU^{24} was followed up by the irritant dose 50 on NMRI mice, read 24

h after application (ID_{50} in nmol/ear). For convenience of comparison of irritant activities the ID_{50}^{24} frequently is expressed as irritancy: $I_{50} = 1/ID_{50}^{24}$ $nmol^{-1}$. As standard irritant, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was used, exhibiting $IU^{24} = 0.05$ μg/ear, $ID_{50} = 0.016$ nmol/ear or $I_{50}^{24} = 63$ $nmol^{-1}$.

SYSTEMATIC FRACTIONATION PROCEDURES.—The separation procedure of latex of *E. ledienii* monitored by IU in main and side stream fractions is fully described (Scheme 1). The latices of the other species were fractionated in an analogous manner. In view of the IU^{24} of TPA, fractions with an IU > 1000 μg/ear are considered inactive. For an overview of the data of isolated *Euphorbia* factors or substances see Table 1. All yields recorded in Table 1 reflect minimum amounts of Euphorbia factors or substances contained in the dry residues of the $(Me)_2CO$ extract obtained from the latex preparations entered into the systematic, quantitative separation procedure (e.g., Scheme 1).

LATEX OF *E. LEDIENII*.—The latex preparation (800 ml) was extracted three times with Me_2CO (Scheme 1). By partitioning of the Me_2CO extract in EtOAc/H_2O, the EtOAc extract (71%) and a hydrophilic fraction (29%) were obtained. The latter was distributed with *n*-BuOH yielding the *n*-BuOH extract (8.4%). The EtOAc extract was subjected to Craig distribution I (n = z = 30 elements, upper phase/lower phase V = 100 ml/100 ml, fundamental procedure) in petroleum ether-MeOH-H_2O (15:10:0.5) to obtain the hydrophobic (62%) and hydrophilic (9%) fractions. The hydrophilic fraction was subjected in the system above to Craig distribution II employing an automatic battery (z = 1000 elements, upper phase/lower phase V = 5 ml/3 ml, single withdrawal procedure by n = 2250 transfers yielding fractions r = 0–2250). Guided by the distribution diagram and assisted by tlc tests, appropriate sequences of fractions r were combined to yield sections 1–17 (Scheme 1). From the active sections 1–5 the *Euphorbia* factors Le_1–Le_4 and Le_1'–Le_5' and from the nonactive sections 6–17 the *Euphorbia* substances SLe_1, SLe_2, and SLe_1'–SLe_3' were isolated by tlc and hplc (Table 1, Scheme 1).

LATEX OF *E. COERULESCENS*.—Latex preparation (100 ml) was worked up in the same manner as the latex of *E. ledienii*. After multistage Craig distribution from the active sections, the *Euphorbia* factors were isolated by tlc and characterized. The factors Le_3, Le_3', and Le_5' show spectroscopic data identical to those obtained for these factors isolated from *E. ledienii* (Table 1). An additional unknown factor Coe_2 will be described below.

LATEX OF *E. TRIANGULARIS*.—Latex preparation (250 ml) was worked up in the same way as the latex of *E. ledienii*. After multistage Craig distribution from the active sections, the *Euphorbia* factors were isolated by tlc. Seven of the irritants isolated show the same spectroscopic data as the factors Le_1, Le_2, Le_4, and Le_1'–Le_4' isolated from *E. ledienii* (Table 1). An additional unknown *Euphorbia* factor T_4 will be described below (20).

CHARACTERIZATION OF THE DITERPENE ESTERS ISOLATED.—For nmr spectroscopic characterization a representative of each structural type is described in detail. Other compounds of the same type showed very similar nmr spectra, reflecting the presence of an identical diterpene moiety and pattern of substitution. Identification of the acid moieties was done easily by comparing the non-diterpene part of the nmr spectra with the spectra of the respective acid methyl esters taken from a spectra catalog.

ESTERS OF 12-DEOXYPHORBOL [**1**].—*12-Deoxyphorbol-13-isobutyrate* [**2**].—Each of the sections 1, 2, and 3 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (5:1) (H_2O saturated) and hplc [column RP-18, 4.6 mm × 25 cm; eluent MeOH-H_2O (80:20)]. Euphorbia factor Le_1 [**2**] was isolated in a total amount of 95 mg (0.17%). Ms *m/z* $[M]^+$ 418, 330; ^1H nmr 7.60 (m, H-1), 5.71 (d, $J = 6$ Hz, H-7), 4.03 (s, H_2-20), 3.29 (m, H-10), 3.03 (m, H-8), 2.49 (m, H_2-5), 1.78 (m, H_3-19), isobutyrate ca. 2.8–2.2 (m, superimposed), 1.3–1.1 ppm (d, 7.5 Hz); ir (KBr) 3420 (OH), 1710 cm^{-1} (C=O); uv (MeOH) λ max (ε) 204 (sh) (9490), 234 (4530), 330 nm (110).

12-Deoxyphorbol-13-tiglate [**3**].—Section 2 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (5:1) (H_2O saturated), developed twice, and the *Euphorbia* factor Le_2 [**3**] isolated in an amount of 41 mg (0.075%). Ms *m/z* $[M]^+$ 430, 330; ^1H nmr tiglate 6.89 (m), 1.90–1.75 ppm (m, $2H_3$, superimposed by H_3-19); ir (KBr) 3410 (OH), 1700 cm^{-1} (C=O); uv (MeOH) λ max (ε) 210 (15710), 224 nm (sh) (13800).

12-Deoxyphorbol-13-(2-methylbutyrate) [**4**].—Section 3 (*E. ledienii*) was separated by hplc using the same conditions as for compound **2** and section 2 by tlc in Et_2O-petroleum ether (5:1) (H_2O saturated). The *Euphorbia* factor Le_3 [**4**] was isolated in a total amount of 294 mg (0.54%). Ms *m/z* $[M]^+$ 432, 330; ^1H nmr 2-methylbutyrate 2.4 (m), 1.2 (CH_2), 1.02 (d, Me), 0.85 ppm (t, Me); ir (KBr) 3420 (OH), 1710 cm^{-1} (C=O); uv (MeOH) λ max (ε) 196 (10990), 233 (5180), 258 nm (sh) (3540).

12-Deoxyphorbol-13-acetate [**5**].—The *E. triangularis* section was separated by tlc in Et_2O-petroleum

ether (3:1). The crude **5** subsequently was purified in $CHCl_3$-MeOH (20:1) and the *Euphorbia* factor T_4 [**5**] isolated in a total amount of 2 mg (0.003%). Ms *m/z* $[M-18]^+$ 372, 330; ^1H nmr acetate 2.09 ppm (δ).

12-Deoxyphorbol-13-isobutyrate-20-acetate [**6**].—Section 4 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (4:1). The band R_f 0.42–0.50 was purified by hplc using the same conditions as for compound **2** and the *Euphorbia* factor Le_1' [**6**] isolated in an amount of 12 mg (0.022%). Ms *m/z* $[M]^+$ 460, 372, 330; ^1H nmr 7.58 (m, H-1), 5.71 (d, $J=6$ Hz, H-7), 4.46 (s, H_2-20), 3.27 (m, H-10), 2.99 (m, H-8), 2.46 (d, $J=3$ Hz, H_2-5), 1.78 (m, H_3-19), acetate 2.04 (s), isobutyrate ca. 2.7–2.3 (m, superimposed), 1.25–1.05 ppm (superimposed); ir (KBr) 3420 (OH), 1740, 1720 cm^{-1} (C=O); uv (MeOH) λ max (ε) 198 (11830), 235 nm (sh) (4680).

Partial transesterification of **6**.—$HClO_4$/MeOH (0.3%) (1.5 ml) was added to **6** (1.5 mg). After 20 h, phosphate buffer pH 7 was added. The solution was extracted three times with EtOAc. After purification by tlc in Et_2O-petroleum ether (4:1), **2** could be isolated (1 mg). Ms *m/z* $[M]^+$ 418; ^1H-nmr signals identical with those reported for **2**.

12-Deoxyphorbol-13-tiglate-20-acetate [**7**].Section 4 (*E. ledienii*) was separated using the same conditions as for compound **6**, and the *Euphorbia* factor Le_2' [**7**] was isolated in an amount of 15 mg (0.027%). Ms *m/z* $[M]^+$ 472, 372, 330; ^1H nmr acetate 2.05, tiglate 6.88 (m), 1.85–1.70 ppm (m, $2H_3$, superimposed by H_3-19).

12-Deoxyphorbol-13-(2-methylbutyrate)-20-acetate [**8**].—Section 5 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (1:1) (H_2O saturated). The crude **8** subsequently was purified in $CHCl_3$-EtOH (95:5), and the tlc-uniform *Euphorbia* factor Le_3' [**8**] was isolated. This factor was also obtained from section 4 (*E. ledienii*) by hplc in the same manner as for compound **2**; total amount 79 mg (0.14%). Ms *m/z* $[M]^+$ 474, 414, 330; ^1H nmr acetate 2.06 (s), 2-methylbutyrate 2.4 (m), 1.18 (CH_2), 1.08 (d, Me), 0.92 ppm (t, Me).

ESTERS OF 12-DEOXY-16-HYDROXYPHORBOL [**9**].—*12-Deoxy-16-hydroxyphorbol-13-angelate-16-isobutyrate* [**10**].—Each of the sections 1 and 2 (*E. ledienii*) were separated by tlc in Et_2O-petroleum ether (5:1) (H_2O saturated), and *Euphorbia* factor Le_4 [**10**] was isolated in a total amount of 51 mg (0.093%). Ms *m/z* $[M]^+$ 516; ^1H nmr 7.59 (m, H-1), 5.66 (d, $J=6$ Hz, H-7), 4.15 ± 0.21 (AB system, $J_{AB}=11$ Hz, H_2-16), 4.02 (s, H_2-20), 3.30 (m, H-10), 3.07 (m, H-8), 2.53 (m, H_2-5), 1.82 (m, H_3-19), 0.95 (d, $J=6$ Hz, H_3-18), angelate 6.08 (m), 2.1–1.9 (2 m), isobutyrate ca. 2.7–2.3 (m, superimposed), 1.3–1.1 ppm (superimposed); ir (CD_2Cl_2) 3400 (OH), 1705 cm^{-1} (C=O); uv (MeOH) λ max (ε) 201 (16110), 224 (sh) (11700), 260 nm (sh) (3260).

12-Deoxy-16-hydroxyphorbol-13-angelate-16-(2-methylbutyrate) [**11**].—The *E. coerulescens* section was separated by tlc in Et_2O-petroleum ether (1:1) and the *Euphorbia* factor Coe_2 [**11**] isolated. Ms *m/z* $[M]^+$ 530; ^1H nmr angelate 6.08 (m), 2.10–1.85 (2m, superimposed), 2-methylbutyrate 2.4 (m), 1.20 (CH_2), 1.02 (d, Me), 0.89 ppm (t, Me).

12-Deoxy-16-hydroxyphorbol-13-angelate-16-isobutyrate-20-acetate [**12**].—Each of the sections 3 and 4 (*E. ledienii*) was separated by hplc using the same conditions as for compound **2**, and the *Euphorbia* factor Le_4' [**12**] was isolated in a total amount of 86 mg (0.16%). Ms *m/z* $[M]^+$ 558; ^1H nmr 7.60 (m, H-1), 5.70 (d, $J=6$ Hz, H-7), 4.44 (s, H_2-20), 4.14 ± 0.20 (AB system, $J_{AB}=11$ Hz, H_2-16), 3.27 (m, H-10), 3.06 (m, H-8), 2.44 (m, H_2-5), 1.79 (m, H_3-19), 0.89 (d, $J=6$ Hz, H_3-18), acetate 2.07 (s), angelate 6.08 (m), 2.10–1.85 (2m, superimposed), isobutyrate 2.7–2.3 (m, superimposed), 1.25–1.10 ppm (superimposed); ir (KBr) 3420 (OH), 1710 cm^{-1} (C=O); uv (MeOH) λ max (ε) 205 (16240), 257 (sh) (4390), 333 nm (90).

Partial transesterification of **12**.—Compound **12** (4.6 mg) was transesterified in the same manner as for compound **6**, and 2 mg of **10** was isolated by tlc in Et_2O-petroleum ether (4:1). Ms *m/z* $[M]^+$ 516; ^1H-nmr signals identical with those reported for **10**.

12-Deoxy-16-hydroxyphorbol-13-angelate-16-(2-methylbutyrate)-20-acetate [**13**].—Section 4 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (4:1) and by hplc using the same conditions as for compound **2**, and the *Euphorbia* factor Le_5' [**13**] was isolated in a total amount of 37 mg (0.067%). Ms *m/z* $[M]^+$ 572; ^1H nmr acetate 2.06 (s), angelate 6.07 (m), 2.10–1.85 (2 m, superimposed), 2-methylbutyrate 2.52 (m, superimposed), 1.20 (CH_2), 1.14 (d, Me), 1.06 ppm (t, Me); ir (KBr) 3420 (OH), 1710 cm^{-1} (C=O); uv (MeOH) λ max (ε) 205 (18220), 252 (sh) (4650), 333 nm (80).

ESTERS OF INGOL [**14**].—*Ingol-7,8,12-acetate, ditiglate* [**15**].—Section 6 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (1:1), and SLe_1 [**15**] was isolated in an amount of 3 mg (0.006%). Ms *m/z* $[M]^+$ 572; ^1H nmr 5.67 (m, H-5), 5.34 (m, H-7), 4.89 (dd, $J_1=4$ Hz, $J_2=10$ Hz, H-12), 4.70 (m, H-8), 4.29 (d, $J=8$ Hz, H-3), acetate 2.16–2.10 (2 s, H_3-20, MeCO), tiglate 6.86 ppm (m).

Ingol-3,7,8-acetate, 2-methylbutyrate, tiglate [**16**].—Section 13 (*E. ledienii*) was separated by tlc in Et_2O-petroleum ether (2:1), and SLe_2 [**16**] was isolated in an amount of 8 mg (0.015%). Ms *m/z* $[M]^+$ 574; ^1H nmr 5.63 (m, H-5), 4.49 (dd, $J_1 = 4$ Hz, $J_2 = 10$ Hz, H-12), 3.21 (dd, $J_1 = 3$ Hz, $J_2 = 10$ Hz, H-8), 2.1 (m, H_3-20), acetate 2.02 (s), 2-methylbutyrate 2.4 (m), 1.26 (CH_2), 1.02 (d, Me), 0.85 (t, Me), tiglate 6.88 ppm (m).

Ingol-3,7,8,12-diacetate, ditiglate [**17**].—Section 14 and the combined sections 11 and 12 (all *E. ledienii*) were separated by tlc in Et_2O-petroleum ether (2:1), and SLe_1' [**17**] was isolated in a total amount of 72 mg (0.13%). Ms *m/z* $[M]^+$ 614; ^1H nmr acetate 2.15–2.00 (2s, H_3-20/MeCO), tiglate 6.87 ppm (m).

Ingol-3,7,8,12-diacetate, (2-methylbutyrate), tiglate [**18**].—The combined sections 15–17 (all *E. ledienii*) were separated by tlc in Et_2O-petroleum ether (2:1), and SLe_2' [**18**] was isolated in a total amount of 20 mg (0.037%). Ms *m/z* $[M]^+$ 616; ^1H nmr acetate 2.01 (s), 2-methylbutyrate 2.6 (m), 1.18 (CH_2), 1.02 (d, Me), 0.86 (t, Me), tiglate 6.87 ppm (m).

Ingol-3,7,8,12-triacetate, tiglate [**19**].—The combined sections 9 and 10 (*E. ledienii*) were separated by tlc in Et_2O-petroleum ether (2:1), and SLe_3' [**19**] was isolated in an amount of 47 mg (0.086%). Ms *m/z* $[M]^+$ 574; ^1H nmr acetate 2.01 (s, 3-MeCO), tiglate 6.87 ppm (m).

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