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TUMOR-PROMOTING COMPOUNDS FROM EUPHORBIA COOPERI
DI- AND TRIESTERS OF 16-HYDROXY-12-DESOXY-PHORBOL.

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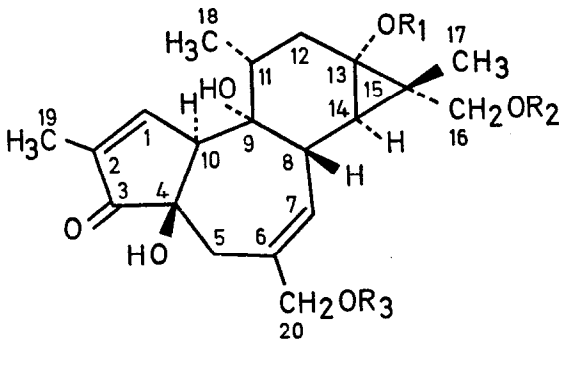
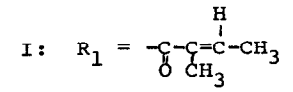
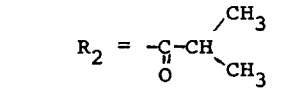
By a combination of liquid-liquid extraction methods with adsorption chromatography from latex of Euphorbia cooperi[+)] two new irritant and tumor promoting compounds C (I), C' (II) have been isolated.

The mass spectra of C (M^+516) and C' (M^+558) show that they are di- and triesters respectively of the same parent alcohol (III: mw = 364). Further, the fragmentations M^+ - 88 and M^+ - 100 in the diester C and M^+ - 60, M^+ - 88 and M^+ - 100 in the triester C' indicate that in both compounds the estergroups may contain the same C_4- (88) and C_5-acid with one C=C-double bond (100) respectively in addition to acetic acid (60) in the triester C'. Acid catalysed selective transesterification ($HClO_4$/MeOH) of the acetic acid ester group in C' yields C.



I: R_1 = 

R_2 = 

R_3 = H

II: R_1, R_2 as in I; R_3 = 

III: $R_1 = R_2 = R_3 = H$

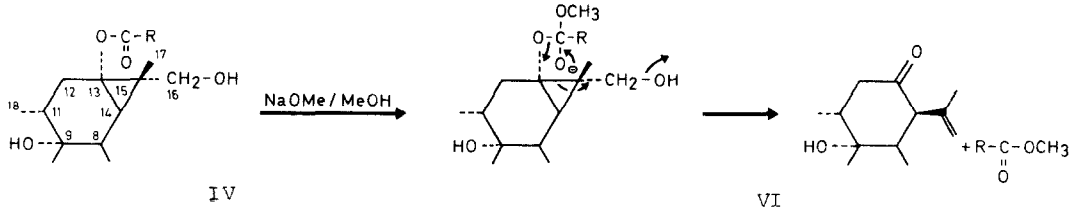
IV: R_1 as in I; $R_2 = R_3 = H$

V: R_1 as in I; $R_2 = R_3$ = 

+) We are greatly indebted to Dr.R.Dyer amd Mr.G.Vahrmeijer,Department of Agricultural Technical Services, Botanical Research Institute, Pretoria, Republic of South Africa,for supply of latex.

By analysis of the nmr-spectrum of I the ester groups in this triester may be further characterized and identified: the sharp singlet at 2,o3 ppm is characteristic of an acetyl group, the doublet (J = 7 Hz) at 1,13 ppm of the geminal methylgroups of an isobutyric acid residue (C_4-acid). The CH_3-peaks at 1,91 and 1,96 ppm show the third acid residue to be tiglic acid (C_5-acid with one C=C-double bond). The couplings (J_1=7 Hz, J_2=1 Hz) of these CH_3-signals with the =CH-signal at 6,o7 ppm can be shown by double resonance experiments.

Base catalysed transesterification ($NaOCH_3$/MeOH) from I yields a monotigliate IV. Trials to obtain the parent alcohol failed, instead by a rapid reaction IV (R_F o,13, thin layer, Silicagel b/e[+]=1/3) is converted to a less polar compound (R_F o,37 system as IV). Acetylation of IV with Ac_2O/pyridine yields a monotigliate-diacetate (V; M^+ 53o) with an uv-spectrum $\lambda_{max}^{CH_3OH}$ (245), 33o nm (ε 56oo,7o) very similar to phorbol-12,13,2o-triacetate (1). Also the nmr-spectrum of V (chart 1, table 1) shows most of the signals of phorbol-12,13,2o-triacetate with similar chemical shifts and multiplicities (1,2). However, the signal of one of the CH_3-groups at the cyclopropanering (CH_3-16 or 17) as well as the signal of the -CH(OAc) group (H-12) as exhibited by phorbol-12,13,2o-triacetate are not present in V. Furthermore a new signal of a $-CH_2$(OAc) group at 4,16 ppm and of a $-CH_2$ group at 2,1 ppm is observed in V. These results suggest that the parent alcohol of I and II is an isomer of phorbol with OH-12 translocated from position 12 to either one of the methylgroups in position 16 or 17 (see III).



IV VI

Compound R_F o,37 obtained in trials to obtain III from IV (see above) is identical with crotophorbolon (VI) by melting point, mixed melting point, ir-spectra and α_D^{2o} = 173° (1% in EtOH). VI is one of the products of the reaction of phorbol

+) b = benzene, e = ethylacetate

with o,o2 n sulfuric acid (2,3). The transformation of IV to crotophorbolon (VI) may be understood as base catalysed elimination of OH-16 (see scheme). It thus proves the structure of the parent alcohol III as derived from nmr-measurements including the absolute configurations at seven out of eight asymmetric centres at C-4,8,9,Io,11,13,14. Thus III is a 4,9,13,16(or 17),2o-Pentahydroxy-tiglia-dien-1,6-on-(3). The absolute configuration at the eighth asymmetric centre (at C-15) may be judged from sterical considerations using Dreiding models. With the $-CH_2OCOCH_3$ group of V in ß-(endo)-position a strong intramolecular H-bridge from the estercarbonyl to OH-4 may be expected. Because the position of the signal of OH-4 (by D_2O-exchange) in the nmr-spectra of V and of phorbol-12,13,2o-triacetate is identical OH-4 does not seem to participate in a H-bridge. Therefore most probably the $-CH_2OCOCH_3$ group in V and the $-CH_2OH$-group in III are in α-(exo)-position,i.e. cis-configuration with respect to the reference atom H-14. From the point of view of diterpene biosynthesis too an exo-position of $-CH_2OH$ at C-15 is more likely: usually the straight chain precursor geranyl-geraniol-pyrophosphate is cyclised first and the resulting product subsequently oxygenated. In a corresponding perhydroazulene precursor of phorbol (4) the methylgroup 17 in ß-or endo-position [+)] would not be as easily accessible for hydroxylation as the methylgroup 16 in α-or exo-position. Therefore III is most probably 4,9,13,16,2o-Pentahydroxy-tiglia-dien-1,6-on-(3).

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+) Of both methylgroups in 15-position the ß-methyl was defined as number 17 (5).

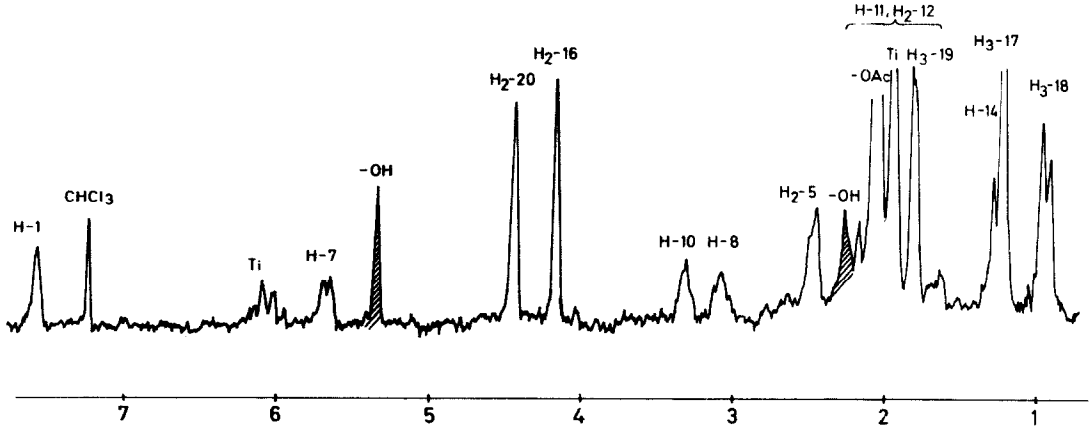

Chart 1: 100 MHz nmr-spectrum of the monotigliate-diacetate (V) in $CDCl_3$ with tetramethylsilane (δ = 0,00 ppm) as internal standard. Ti = signals of the tiglic acid residue.

Table 1: Decoupling in the nmr-spectrum of V by double resonance experiments.

irradiated at ppm	observed at ppm		change in multiplicity	removed coupling (J in Hz)
H-1	7,56	H_3-19	1,80	dd ⟶ d	<1
H-10	3,32	H_3-19	1,80	dd ⟶ s (broad)	2
H-10	3,32	H-1	7,56	sharpening	-
H-7	5,66	H-8	3,06	t ⟶ d	5,5
H-7	5,66	H_2-5	2,46	sharpening	-
H-8	3,06	H-7	5,66	d ⟶ s	5,5
H-8	3,06	H-14	1,25	d ⟶ s	5
H-14	1,25	H-8	3,06	t ⟶ d	5
H-11	2,06	H_3-18	0,92	d ⟶ s	6