New highly irritant euphorbia factors from latex of Euphorbia tirucalli L.

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Summary. From the latex of Euphorbia tirucalli L. growing in Madagascar, 5 new euphorbia factors were isolated. They were characterized as 13-O-acetyl-12-O-acylphorbol- and 12-O-acetyl-13-O-acylphorbol derivatives carrying homologous conjugated unsaturated fatty acids as acyl groups. Furthermore, 2 mixtures of homologous 3-O-acylingenol derivatives are obtained carrying the same type of unsaturated fatty acids. Due to their highly unsaturated acyl groups all Euphorbia factors or factor groups isolated are highly sensitive to autoxidation.

Euphorbia tirucalli L. is an almost leafless succulent shrub or tree ('pencil tree') wide spread throughout all tropical regions of the world[2]. The use of E. tirucalli L. for medical purposes, as fish poison and as insecticide and the highly vesicant and irritant properties of the latex towards the skin and to mucous membranes are well known[3]. Most recently, large scale plantation of E. tirucalli L. was propagated in connection with investigations of future resources of gasoline[4].

The acetone extract of the latex of E. tirucalli L. collected in Madagascar was subjected to a series of O'Keeffe distributions to obtain 2 highly irritant ester fractions I and II (for assays see[5]). Base catalyzed transesterifications of fraction II yields phorbol (**1**) and of fraction I phorbol (**1**) and ingenol (**4**), characterized as 12, 13,20-tri-O-acetylphorbol (**2**)[6] and 3,5,20-tri-O-acetylingenol (**5**)[7], respectively. By combination of Craig distribution with adsorption chromatography under strict exclusion of oxygen from fraction II 4 highly irritant euphorbia factors Ti_5-Ti_8 were isolated. Similarly, from fraction I a further highly irritant euphorbia factor Ti_9 and 2 irritant factor groups Ia and Ib were obtained. Ti_5-Ti_9 were all shown to contain phorbol (**1**) and Ia and Ib to contain ingenol (**4**), respectively, and represent a new class of irritant diterpene esters in carrying homologous conjugated unsaturated acyl residues (for factors Ti_1-Ti_4 see[8]).

Ti_5: MS m/e: 528 (M^+), 468 (M^+-60), 389 (M^+-139), 123 (base peak); IR (KBr): ν_{max}: 3420, 1740, 1720, 1640, 1605, 995, 965 cm^{-1}; UV (CH_3OH): λ (ε): 193 nm (14750); λ_{max} (ε_{max}): 205 (12630), 262.5 nm (23230); ^{1}H-NMR (δ, $CDCl_3$; see also chart 1): 7.58 (s, broad, 1-H), 7.35 (dd, $J_{4',5'}$ = 16Hz, $J_{4',3'}$ = 11 Hz, 4'-H), 6.56 (dd, $J_{3',4'} = J_{2',3'}$ = 11 Hz, 3'-H), 6.06 (m, 5'-H), 5.66 (d, J = 7 Hz, 7-H), 5.50 (d, $J_{2',3'}$ = 11 Hz, 2'-H), 5.42 (d, J = 10 Hz, 12-H), 4.00 (s, 20-H_2), 3.26 (m, 8-H), 3.26 (m, 10-H), 2.53 (s, 5-H_2), 2.17 (m, 6'-H_2), 2.11 (s, CH_3CO), 1.75 (m, 19-H_3), 1.24, 1.20 (s, 16-H_3, 17-H_3), 0.87 (d, J = 6 Hz, 18-H_3), 5.67, 2.95, 2.2 ppm (OH, exchangeable).

According to these data, Ti_5 contains an acetyl- and an 2,4-octadienoyl group: The latter is confirmed by the base peak m/e = 123 ($C_8H_{11}O$, high res. MS) arising from the corresponding acyloxy radical m/e 139. It lacks 4 hydrogen atoms in comparison to octanoic acid, which was obtained as methyl ester after base catalyzed transesterification in methanol of Ti_5 and catalytic hydrogenation of the methyl ester acquired (see table 1). The stereochemistry of the double bonds is determined by double resonance experiments: 2'-H appears at 5.50 ppm as doublet ($J_{2',3'}$ = 11 Hz) corresponding to the 2',3'-cis double bond[9]; 4'-H is found at 7.35 ppm as doublet of doublet ($J_{4',5'}$ = 16 Hz, $J_{3',4'}$ = 11 Hz) corresponding to the 4',5'-trans double bond and to the coupling of 3'-H and 4'-H.

Selective transesterification of Ti_5 yields the 12-monoester of Ti_5: MS (m/e): 486 (M^+), 346 (M^+-140); ^{1}H-NMR (δ,$CDCl_3$) with the signal of the acetyl group abolished and the doublet of 12-H shifted to 4.85 ppm (table 2). This upfield shift of the signal of the vicinal C-12 proton upon transesterification of a 13-acetate is in accordance with earlier observations[10]. It confirms the assignment of the (2Z,4E)-2,4-octadienoic acid residue to position 12.

Acetylation of Ti_5 with acetic anhydride/pyridine yields the 20-acetate **3**, MS (m/e): 570 (M^+); ^{1}H-NMR (δ, $CDCl_3$) with the signal of 20-H_2 shifted downfield (s, 4.46 ppm) and the signal of one newly introduced acetyl group at 2.04 ppm. Thus, by exclusion, Ti_5 is 13-O-acetyl-12-O-[(2Z,4E)-2,4-octadienoyl] phorbol (see structural formula).

In a similar way, the rest of the new euphorbia factors were structurally elucidated. Ti_6: MS: (m/e) 554 (M^+), 494 (M^+-60), 389 (M^+-165); IR (KBr): ν_{max}: 3420, 1740, 1720, 1640, 1620, 1585, 1005, 975 cm^{-1}; UV (MeOH): λ (ε): 193 nm (15440): λ_{max} (ε_{max}): 205 (13600), 304 nm (24800); ^{1}H-NMR (δ, $CDCl_3$): differences to the spectrum of Ti_5: 7.37 (dd, $J_{4',5'}$ = 16 Hz, $J_{3',4'}$ = 11 Hz), 5.60 (d, $J_{2',3'}$ = 11 Hz), between 7.0 and 5.8 ppm 4 olefinic protons of the acid residue. According to these data and those given in tables 1 and 2, Ti_6 is 13-O-acetyl-12-O-[(2Z,4E)-2,4,6-decatrienoyl] phorbol. The comparison of our data with those of the piscicidal 13-O-acetyl-12-O-(2,4,6-decatrienoyl)phorbol of Sapium japonicum[11] shows that the unsaturated acid residue in the latter is most probably also (2Z,4E)-2,4,6-decatrienoic acid.

Ti_7: MS: (m/e) 606 (M^+), 546 (M^+-60), 389 (M^+-217); IR (KBr): ν_{max}: 3425, 1740, 1720, 1630, 1585, 1000 cm^{-1}; UV (MeOH): λ (ε): 194 nm (17770); λ_{max} (ε_{max}): 204 (18240) 227 (11850), 260 (13090), 357 nm (44580); ^{1}H-NMR (δ, $CDCl_3$): differences as compared to Ti_5: between 7.5

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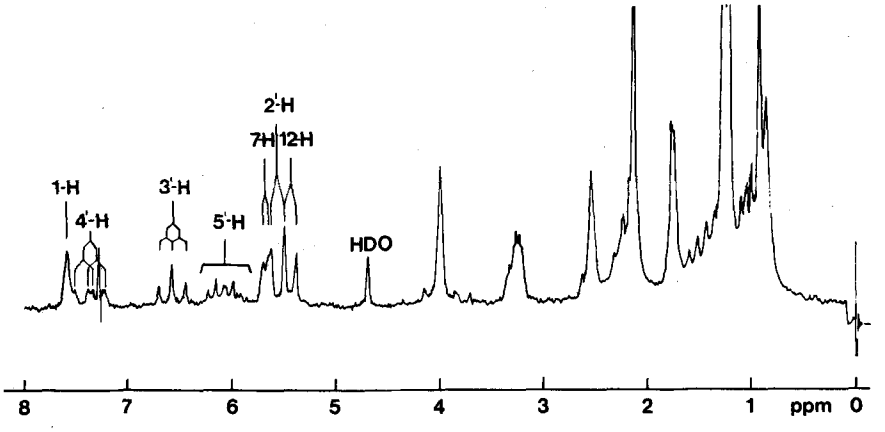
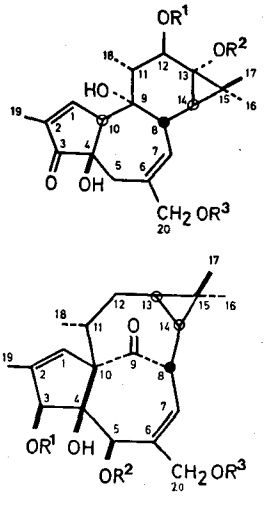


Chart 1. ^{1}H-NMR-spectrum of Ti_5, 90 MHz, $CDCl_3/D_2O$, TMS (δ = 0.00 ppm).



1 : $R^1=R^2=R^3=H$

2 : $R^1=R^2=R^3=Ac$

3 : R^1=CO-CH$\overset{Z}{=}$CH-CH$\overset{E}{=}$CH-CH_2-CH_2-CH_3; $R^2=R^3=Ac$

Ti_5: R^1=CO-CH$\overset{Z}{=}$CH-CH$\overset{E}{=}$CH-CH_2-CH_2-CH_3 (1' 2' 3' 4' 5' 6' 7' 8'); $R^2=Ac$; $R^3=H$

Ti_6: R^1=CO-CH$\overset{Z}{=}$CH-CH$\overset{E}{=}$CH-CH=CH-CH_2-CH_2-CH_3 (1' 2' 3' 4' 5' 6' 7' 8' 9' 10'); $R^2=Ac$; $R^3=H$

Ti_7: R^1=CO-(CH=CH)$_5$-CH_2-CH_2-CH_3; $R^2=Ac$; $R^3=H$

Ti_8: R^1=CO-(CH=CH)$_4$-$(CH_2)_4$-CH_3; $R^2=Ac$; $R^3=H$

Ti_9: $R^1=Ac$; R^2=CO-(CH=CH)$_5$-CH_2-CH_2-CH_3; $R^3=H$

4 : $R^1=R^2=R^3=H$; 5 : $R^1=R^2=R^3=Ac$

6 : $R^1=H$; $R^2=R^3=C(CH_3)_2$

1a : R^1=CO-(CH=CH)$_n$-CH_2-CH_2-CH_3 n=3,4,5; $R^2=R^3=H$

1b : R^1=CO-(CH=CH)$_n$-$(CH_2)_4$-CH_3 n=2,3,4; $R^2=R^3=H$

Table 1. Characterization of unsaturated fatty acids

Euphorbia factor UV: λ_{max} nm (ε_{max})		Fatty acid or methyl ester MS: M^+ (m/e)	UV: λ_{max} nm (ε_{max})	Hydrogenated fatty acid methyl ester GLC (according to authentic reference)
Ti_5	262.5 (23230)	140	258 (–)	Octanoate
Ti_6	304 (24800)	180	306 (–)	Decanoate
Ti_7	357 (44580)	232	259 (7350) 357 (44580)	Tetradecanoate
Ti_8	336 (36000)	234	– (–)	Tetradecanoate
Ti_9	349 (29000)	232	260 (6400) 352 (30850)	Tetradecanoate

Table 2. MS- and NMR-data relevant for the position of the ester residues in Ti_5-Ti_9

Euphorbia factor (12, 13-diester)					Corresponding 12-monoester		
	MS	NMR ($CDCl_3/D_2O$, TMS, δ = 0.00 ppm)			MS	NMR ($CDCl_3/D_2O$, TMS, δ = 0.00 ppm)	
	M^+ (m/e)	12–H	20–H	CH_3CO	M^+ (m/e)	12–H	20–H
Ti_5	528	5.42	4.00	2.11	486	4.85	4.00
Ti_6	554	5.44	4.00	2.10	346 (M^+–166)	4.88	4.00
Ti_7	606	5.46	4.00	2.10	346 (M^+–218)	4.90	4.00
Ti_8	608	5.48	4.00	2.10	566	4.86	4.00
Ti_9	606	5.55	4.04	2.12	406	4.82	3.98

and 5.5 ppm 10 olefinic protons of the acid residue. The preceding spectral data and those given in tables 1 and 2 prove that Ti_7 is 13-O-acetyl-12-O-(2,4,6,8,10-tetradecapentaenoyl)phorbol.

Ti_8: MS: (m/e) 608 (M^+), 548 (M^+-60), 389 (M^+-219); IR (KBr): ν_{max}: 3430, 1715, 1625, 1605, 1000 cm^{-1}; UV (CH_3OH): λ (ε): 193.5 nm (17680); λ_{max} (ε_{max}): 203.5 (16200), 231 (10530), 336 nm (35000); ^{1}H-NMR (δ, $CDCl_3$): differences as compared to Ti_5: between 7.4 and 5.5 ppm 8 olefinic protons of the acid residue. In accordance with the preceding data and those given in tables 1 and 2 Ti_8 is 13-O-acetyl-12-O-(2,4,6,8-tetradecatetraenoyl)phorbol.

Ti_9: MS: (m/e) 606 (M^+), 546 (M^+-60), 389 (M^+-217); UV (MeOH): λ_{max} (ε_{max}): 260 (9000), 394 nm (29000); ^{1}H-NMR (δ, $CDCl_3$): differences as compared to Ti_5: slight downfield shifts of 12-H at 5.55 ppm and OH-9 at 5.67 ppm; between 7.5 and 5.5 ppm 10 olefinic protons of the acid residue. The combination of these data with those given in tables 1 and 2 proves that Ti_9 is 12-O-acetyl-13-O-(2,4,6,8,10-tetradecapentaenoyl)phorbol.

A common feature of the euphorbia factors Ti_5-Ti_9 is their marked instability with respect to autoxidation yielding biologically inactive material insoluble in all solvents. From such materials the parent alcohol may still be obtained by mild hydrolytic procedures.

Factor group Ia (see formula) was separated in 2 subfractions M_{23} and M_{24}. M_{23}: MS (m/e) 522/496 (M^+), 330 (M^+-192/166), 192, 166; ^{1}H-NMR (δ, $CDCl_3$): 7.8-5.7 ppm (7 olefinic protons), all other signals are identical with those of the euphorbia factor I_6 from E. ingens[12]. M_{24}: MS (m/e): 548/522 (M^+), 330 (M^+-218/192), 218, 192; ^{1}H-NMR (δ, $CDCl_3$): differences to the spectrum of M_{23}: 7.8-5.7 ppm (9 olefinic protons of the acid residue). Factor group Ib was separated in 2 subfractions M_{25} and M_{26}, containing 3 further ingenol esters. M_{25}: MS (m/e) 524/498 (M^+), 330 (M^+-194/168), 194, 168; ^{1}H-NMR (δ, $CDCl_3$): differences to the spectrum of M_{23}: 7.8–5.7 ppm (5–6 olefinic protons of the acyl residue). M_{26}: MS (m/e): 550/524 (M^+), 330 (M^+-220/194), 220, 194; ^{1}H-NMR (δ, $CDCl_3$): differences to the spectrum of M_{25}: 7.8–5.7 ppm (7–8 olefinic protons of the acid residue).

Evidence for the ester positions results from NMR-data and from treatment of the 2 factor groups with acetone/$HClO_4$ followed by base catalyzed transesterification in methanol yielding 5,20-O-isopropylideneingenol (**6**)[13]. The chemical structure of the fatty acids follows from the spectral data (MS and UV) of the fatty acid methyl esters obtained by transesterification and from GLC-analysis of the homologous saturated methyl esters, obtained by catalytic hydrogenation.

Thus factor group Ia comprises 3 homologous 3-O-acylingenols, each of which is esterified with a highly unsaturated fatty acid of the type CH_3–$(CH_2)_2$–$(CH=CH)_n$–COOH (n = 3,4,5).3-O-(2,4,6-decatrienoyl)ingenol (n = 3) was isolated already from Euphorbia ingens (euphorbia factor I_6)[12] and 3-O-(2,4,6,8,10-tetradecapentaenoyl)ingenol (n = 5) from latex of E. lathyris (euphorbia factor L_6)[14] and from roots of E. jolkinii Boiss[15]. Factor group Ib comprises 3 homologous 3-O-acylingenols, esterified with highly unsaturated fatty acids of the type CH_3–$(CH_2)_4$–$(CH=CH)_n$–COOH (n = 2,3,4). They were unknown as yet. Again the factor groups Ia and Ib are highly susceptible to autoxidation leading to highly insoluble and biologically inert materials, from which, by hydrolytic procedures, the diterpene parent alcohol may be obtained. The biological data of the new euphorbia factors and factor groups will be published elsewhere.

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β-(p-hydroxyphenyl) ethanol in the chest gland secretion of a galago (Galago crassicaudatus)

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Summary. β-(p-hydroxyphenyl)ethanol is present in the chest gland secretion of the galago Galago crassicaudatus.

Although pheromones have been demonstrated to be of major importance in the social biology of primates[2], the chemistry of these exocrine products still constitutes relative terra *incognita*. Indeed, except for the identification of short chain fatty acids as sexual releasers for Macaca mulatta[3], no primate pheromones have been identified. It has been established, however, that similar acids are produced by other monkeys, baboons, and humans[4]. The purpose of the present note is to report the identification of β-(p-hydroxyphenyl)ethanol in the chest gland secretion of the galago Galago crassicaudatus. This compound releases an unusual behavior response in this primate and may constitute one of its marking pheromones.

Material and methods. The secretion was obtained by wiping the chest gland located on the midline about the base of the throat over the clavick with absorbent tissue. The tissues were immediately extracted with chromatoquality methylene chloride and these extracts were used for all subsequent analyses. Analyses were performed on an LKB-9000 gas chromatograph-mass spectrometer (GC-MS)[5] using a 1% OV-17 column and a 10% SP-1000 column both on Supelcoport 80–100 mesh (Supelco, Bellefont, Pa.). The columns were programmed from 50 °C to 300 °C and 200 °C, respectively.

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