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THE NEW DITERPENE 4-DEOXYPHORBOL AND ITS HIGHLY UNSATURATED IRRITANT DIESTERS

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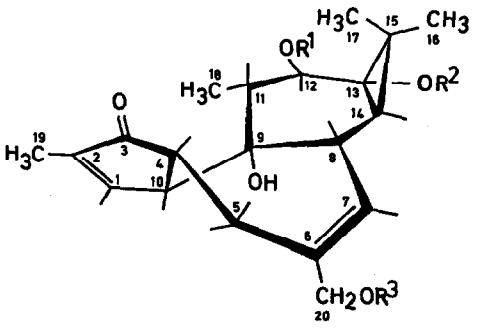
From the latex of Euphorbia tirucalli L. grown in Madagascar a new class of esters of phorbol and ingenol carrying highly unsaturated fatty acids were isolated[1]. In latex of E. tirucalli L. grown in South Africa, besides unsaturated fatty acid esters of phorbol, similar esters of a new diterpene parent were detected. Alternatively, ingenol is present in this latex only in very small amounts.

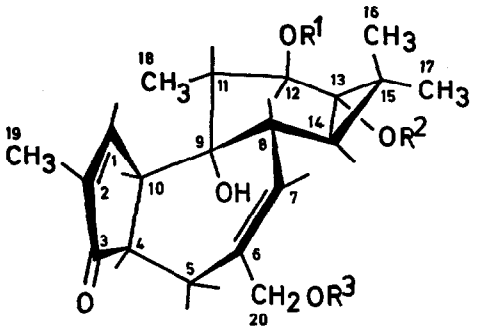
By systematic fractionation[1)2)3)] of the acetone extract under carefully controlled conditions of pH and extensive exclusion of oxygen and monitored by our biological assay for irritant activity[4)] four new highly irritant euphorbia factors Ti_1-Ti_4 were isolated. In addition to Ti_1 and Ti_4, their biologically inactive isomers α-Ti_1 and α-Ti_4 were obtained.

Ti_1: $C_{32}H_{42}O_7$(hrms); MS (m/e): 538 (M^+), 478 (M^+-60), 373 (M^+-165); IR (CH_2Cl_2):ν_{max}: 3670, 3600, 3400, 1715, 1635, 1615, 1585, 1005, 975, 815 cm^{-1}. UV (CH_3OH):λ_{max} (ϵ_{max}): 204 (14050), 227 (8380), 304 nm (26760); ^{1}H-NMR (δ, $CDCl_3$): 7.56 (s, broad, 1-H), 7.3 (m, 4'-H), 7.0-5.7 (4 olefinic protons), 5.59 (d, J=11Hz, 2'-H), 5.53 (m, 7-H), 5.47 (d, J=10Hz, 12-H), 4.00 (s, 20-H_2), 3.26 (m, 10-H), 2.17 (CH_3CO), 1.72 (m, 19-H_3), 1.20 (s, 16-H_3, 17-H_3), 5.67, 2.60 ppm (OH, exchangeable).

α-Ti_1: MS (m/e): 538 (M^+), 478 (M^+-60), 373 (M^+-165); IR (CH_2Cl_2):ν_{max}: 3680, 3600, 3420, 1715, 1645, 1615, 1585, 1005, 975, 815 cm^{-1}; UV (CH_3OH):λ_{max} (ϵ_{max}): 233 (8460), 304 nm (19670); ^{1}H-NMR (δ,$CDCl_3$): characteristic differences to the spectrum of Ti_1 in regard to some protons of the diterpene parent: the broad s of 1-H is shifted upfield to 7.05 ppm, the m of 7-H to 5.13 ppm whereas the signal of 10-H is shifted downfield and appears at 3.51 ppm.

Treatment with $NaOCH_3/CH_3OH$ of Ti_1 and α-Ti_1 and subsequent acetylation with acetic anhydride/pyridine yields in both cases the previously known 12,13,20-tri-O-acetyl-4-deoxy-4α-phorbol (1)[5)]. This result shows that the parent alcohols of Ti_1 and α-Ti_1 are 4-deoxy-derivatives of phorbol. By irradiation with UV-light (λ= 254 nm) of α-Ti_1 followed by base catalyzed transesterification and acetylation with acetic anhydride the lumiproduct 12,13,20-tri-O-acetyl-4-deoxy-lumiphorbol (5)[6)] is obtained. Hence it is proved that the parent alcohol of α-Ti_1 is 4-deoxy-4α-phorbol. In accordance with this structure, the chemical shifts of the signals of 1-H, 7-H and 10-H in nmr-spectra of α-Ti_1 and 12,13,20-tri-O-acetyl-4-deoxy-4α-phorbol (1) are identical. Under the conditions of the conversion of α-Ti_1 to 5, Ti_1 yields 1 but no lumiproduct. The base catalyzed epimerization of Ti_1 to α-Ti_1 in CD_3OD yields 4-deutero-α-Ti_1 (2) as proved by ms-data: 539 (M^+), 479 (M^+-60), 374 (M^+-165) and nmr-data (δ, $CDCl_3$: 7.02 (s, broad, 1-H), 5.17 (m, 7-H), 3.45 ppm (m, 10-H). The signal m of 4-H at 2.7 ppm as present in the nmr-spectrum of α-Ti_1 is missing and the dd ($J_{4,5b}$=5hz, $J_{5a,5b}$=





Ti_1: R^1=CO-CH^Z=CH-CH^E=CH-CH=CH-CH_2-CH_2-CH_3
R^2=Ac; R^3=H

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

Ti_2: R^1=Ac; R^2=CO-CH^Z=CH-CH^E=CH-CH=CH-CH_2-CH_2-CH_3; R^3=H

Ti_3: R^1=Ac; R^2=CO-$(CH=CH)_5$-CH_2-CH_2-CH_3; R^3=H

Ti_4: R^1=Ac; R^2=CO-$(CH=CH)_4$-$(CH_2)_4$-CH_3; R^3=H

α-Ti_1: R^1= CO-CH^Z=CH-CH^E=CH-CH=CH-CH_2-CH_2-CH_3;
R^2=Ac; R^3=H; R^4=H

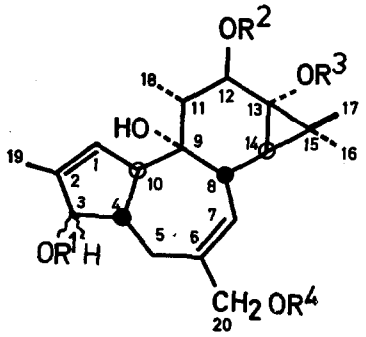
1 : R^1=R^2=R^3=Ac; R^4=H

2 : R^1=CO-CH^Z=CH-CH^E=CH-CH=CH-CH_2-CH_2-CH_3;
R^2=Ac; R^3=H; R^4=D

3 : R^1=CO-CH^Z=CH-CH^E=CH-CH=CH-CH_2-CH_2-CH_3;
R^2=R^3=R^4=H

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

α-Ti_4: R^1=Ac; R^2=CO-$(CH=CH)_4$-$(CH_2)_4$-CH_3;
R^3=R^4=H



5 : R^1=R^2=R^3=R^4=Ac

6 : R^1=H; R^2=R^3=R^4=Ac

15hz) of 5b-H at 2,45 ppm is reduced to a dublet ($J_{5a,5b}$=15hz). This proves that Ti_1 contains 4-deoxyphorbol as diterpene parent. The characteristic differences of the chemical shifts of 1-H, 7-H and 10-H in the nmr-spectra of the epimers Ti_1 and α-Ti_1 are to be understood as shielding effects of the 1,2-and 6,7-double bonds and deshielding of the adjacent 9α-OH respectively, caused by the change of the stereochemistry of the diterpene parent upon epimerization at C-4 (see structural formula)[7].

The (2Z,4E)-2,4,6-decatrienoyl group is confirmed by the spectral data of Ti_1 and α-Ti_1 (ms, uv) and by the ms- and nmr-data of the identical methylesters acquired upon base-catalyzed transesterification of Ti_1 and α-Ti_1: MS (m/e): 180 (M^+); ^{1}H-NMR (δ, $CDCl_3$): 7,35 (dd, $J_{4';5'}$=16Hz, $J_{3';4'}$=11Hz, 4'-H), 6,7-5,6 (4 olefinic protons), 5,54 (d, $J_{2';3'}$=11Hz, 2'-H), 3,66 (s, OCH_3, 3), 2,1 (m,8'-H), 0,90 ppm (t, J=7Hz, 10'-H_3).

Selective transesterification ($NaOCH_3/CH_3OH$) of the acetyl groups in position 13 of both Ti_1 and α-Ti_1 yields 12-O-[(2Z,4E)-2,4,6-decatrienoyl]-4-deoxy-4α-phorbol (3): MS (m/e): 496 (M^+), ^{1}H-NMR (δ, $CDCl_3$, see table 1): 7,05 (s, broad, 1-H) 5.13 (m,7-H), 3.50 ppm (m, 10-H) confirm the 4α-configuration, the doublet of 12-H is shifted upfield to 5.05 ppm in accordance with earlier observations, that the signal of the vicinal 12-H is shifted to higher

field upon hydrolysis of the 13-acetyl or acyl group[8], the signal of the acetyl group is missing. The chemical shift of 20-H_2 remains unchanged. These data confirm the 12-position of the (2Z,4E)-2,4,6-decatrienoyl residue and the 13-position of the acetyl group: Ti_1 is 13-O-acetyl-12-O-[(2Z,4E)-2,4,6-decatrienoyl]-4-deoxyphorbol and α-Ti_1 the corresponding 4-epimer 13-O-acetyl-12-O-[(2Z,4E)-2,4,6-decatrienoyl]-4-deoxy-4α-phorbol.

Table 1: MS- and NMR-data, relevant for the position of the ester residues and the stereochemistry of the diterpene parent in Ti_1-Ti_4, α-Ti_1, α-Ti_4 and the transesterification products 3 and 4 (12-monoesters).

factor/ cpd	MS (m/e) M^+	NMR (δ, $CDCl_3/D_2O$), TMS δ = 0,00 ppm 1-H	7-H	10-H	12-H	20-H	CH_3CO
Ti_1	538	7.56	5.53	3.26	5.47	4.00	2.15
Ti_2	538	7.60	5.52	3.25	5.54	4.00	2.12
Ti_3	590	7.60	-	3.26	5.47	4.02	2.15
Ti_4	592	7.60	-	3.28	5.48	4.02	2.15
α-Ti_1	538	7.05	5.13	3.51	5.54	3.95	2.07
α-Ti_4	592	7.05	5.15	3.50	5.52	3.95	2.09
3	496	7.05	5.13	3.50	5.07	3.95	missing
4	372(M^+-18)	7.05	5.17	3.50	5.05	3.93	2.12

In a similar manner the rest of the new euphorbia factors was structually elucidated.

Ti_2: $C_{32}H_{42}O_7$(hrms):MS(m/e): 538(M^+), 478(M^+-60), 373(M^+-165), 149; IR(CH_2Cl_2): ν_{max}: 3600, 3400, 1705, 1625, 1605, 1575, 1005, 975, 815 cm^{-1}; UV(CH_3OH): λ_{max} (ε_{max}): 230 (9600), 306 nm (25000); ^{1}H-NMR (δ,$CDCl_3$): no characteristic differences to the spectrum of Ti_1. The preceding data and those given in table 1 prove that Ti_2 is 12-O-acetyl-13-O-[(2Z,4E)-2,4,6-decatrienoyl]-4-deoxyphorbol.

Ti_3: MS(m/e): 590 (M^+), 530(M^+-60), 373(M^+-217); IR(KBr): ν_{max}: 3420, 1710, 1640, 1610, 1595, 1575, 1545, 1000 cm^{-1}; UV(CH_3OH): $\lambda(\varepsilon)$: 194 nm (16300); λ_{max} (ε_{max}): 204(15400), 228 (11900), 252(10550), 260(10500), 357 nm (34000); ^{1}H-NMR(δ,$CDCl_3$): differences as compared to Ti_1: between 7.5 and 5.5 ppm 10 olefinic protons of the acid residue. In accordance with the preceding data and those given in table 1 Ti_3 is 12-O-acetyl-4-deoxy-13-O-(2,4,6,8,10-tetradecapentaenoyl)phorbol.

Ti_4: $C_{36}H_{48}O_7$(hrms): MS(m/e): 592(M^+), 532(M^+-60), 373(M^+-219), 203; IR(CH_2Cl_2): ν_{max}: 3600, 3400, 1715, 1695, 1630, 1605, 1590, 1005 cm^{-1}; UV(CH_3OH):λ_{max} (ε_{max}): 230(9530), 332 nm (23000); ^{1}H-NMR(δ,$CDCl_3$): differences as compared to Ti_1: between 7.5 and 5.5 ppm 8 olefinic protons of the acid residue. The combination of these data with those given in table 1 proves that Ti_4 is 12-O-acetyl-4-deoxy-13-O-(2,4,6,8-tetradecatetraenoyl)phorbol.

α-Ti_4: MS(m/e): 592(M^+), 532(M^+-60), 373(M^+-219); UV(CH_3OH): λ_{max} (ε_{max}): 230(9530), 330 nm (19300); ^{1}H-NMR(δ,$CDCl_3$): differences as compared to the spectrum of α-Ti_1: between 7.5 and 5.5 ppm 8 olefinic protons of the acid residue. In confirmity of the preceding data and those given in table 1 α-Ti_4 is 12-O-acetyl-4-deoxy-13-O-(1,4,6,8-tetradecatetraenoyl)-4α-phorbol.

The irreversible epimerization of Ti_1 to α-Ti_1 occurs under very mild basic and acidic conditions [9]. Therefore, the parent alcohol 4-deoxyphorbol cannot be made available directly by transesterification of its naturally occuring esters. It may be obtained by partial synthesis starting with 3,12,13,20-tetra-O-acetyl-3-deoxo-4-deoxy-3(ξ)-hydroxyphorbol (5), the product of reductive scission of Ti_1 with $LiAlH_4$ followed by acetylation with acetic anhydride/pyridine. (5): $C_{28}H_{38}O_9$(hrms); MS(m/e): 518(M^+); IR(KBr): ν_{max}: 3420, 1730 cm^{-1}; UV(CH_3OH): $\lambda(\varepsilon)$: 194 nm (16210); ^{1}H-NMR (δ,$CDCl_3$): 5.80 (s, broad, 1-H), 5.44(d, J=5-6Hz, 7-H), 5.34 (d, J=10Hz, 12-H), 5.18(m, 3-H), 4.42(s, 20-H_2), 3.00(m, 10-H), 2.27 (m, 4-H), 2.24(m, 8-H), 2.10-2.05(4 CH_3CO), 1.62(m, 19-H_3), 1.62(m, 11-H), 1.21(s, 16-H_3, 17-H_3), 0.95(d, J=6Hz, 18-H_3), 0.95(d, J=5-6Hz, 14-H), 5.22 ppm (OH, exchangeable). Treatment of 5 with $HClO_4$/dioxane furnishes 12,13,20-tri-O-acetyl-3-deoxo-4-deoxy-3(ξ)-hydroxyphorbol (6): MS(m/e): 476(M^+); ^{1}H-NMR(δ,$CDCl_3$): differences as compared to (5): the signal of 3-H is found at 4.10 ppm as compared with 5.28 ppm in (5). Oxidation of the free hydroxylfunction 3 in 6 with alkalifree MnO_2/CH_2Cl_2 yields 12,13,20-tri-O-acetyl-4-deoxyphorbol (7): $C_{28}H_{34}O_6$(hrms): MS(m/e): 474 (M^+); IR(KBr): ν_{max}: 3415, 1745, 1730, 1710, 1635 cm^{-1}; UV(CH_3OH): $\lambda(\varepsilon)$: 198,5 nm (11600); λ_{max} (ε_{max}): 230 (6050), 310 nm (140); ^{1}H-NMR(δ,$CDCl_3$): 7.53(s, broad, 1-H), 5.52(dd, J=2Hz, J=7Hz, 7-H), 5.38(d, J=10Hz, 12-H), 4.43(s, 20-H_2), 3.23(m, 10-H), 2.33(m, 4-H), 2.34(m, 8-H), 2.85(dd, J=16Hz, J=6-7Hz, 5a-H), 2.2(m, 5b-H), 2.10, 2.08, 2.05 (CH_3CO), 1.76(m, 19-H_3) 1.57(m,11-H), 1.24, 1.22(s,16-H_3, 17-H_3), 1.05(d, J=5Hz, 14-H), 0.92 (d, J=6Hz, 18-H_3), 5.55 ppm (OH, exchangeable); CD(C_2H_5OH) λ:202, 241, 318 nm$\Delta\varepsilon$: -1943, +3.10, -2.02. The chemical shifts of the protons 1-H, 7-H and 10-H in the nmr spectrum of 7 support the proposed structure of the new diterpene parent of the euphorbia factors Ti_1-Ti_4. The biologically inactive 4-deoxy-4α-phorbol-derivatives α-Ti_1 and α-Ti_4 are products of artificial isomerization during the isolation procedure[9]. Esters of 4-deoxy-4α-phorbol have been isolated from the seed oil of Croton tiglium[4]. - The biological data of the new euphorbia factors will be published elsewhere.

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