

TUMOR PROMOTING COMPOUNDS FROM EUPHORBIA TRIANGULARIS:

MONO- AND DIESTERS OF 12-DESOXY-PHORBOL

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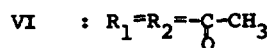
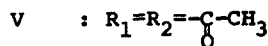
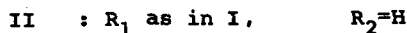
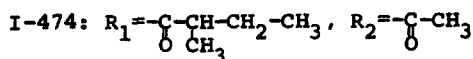
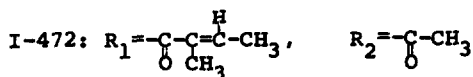
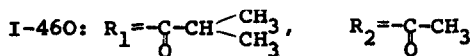
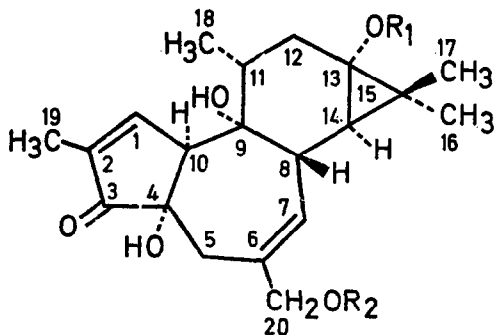
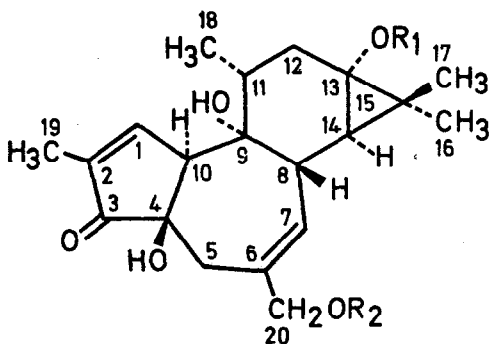
By a combination of multiplicative distribution methods (O'Keeffe and Craig distribution (1)) with adsorption chromatography two irritant (2) and tumor promoting (2) fractions (I,II) have been isolated from the latex of *Euphorbia triangularis*[†].

Mass spectra suggest a mixture of three diesters for fraction I and a mixture of three monoesters for fraction II, both fractions carrying the same parent alcohol III (mw=348). The three diesters (M^+ 460, 472, 474) of fraction I all contain acetic acid (M^+ -60) and as the second acid residue a C_4 - (M^+ 460), a C_5 - (M^+ 474) and a C_5 -acid with one C=C-double bond (M^+ 472) respectively. In the esters of fraction I the acetic acid residue may be removed selectively with $HClO_4/MeOH$ to obtain a mixture of monoesters identical with fraction II (M^+ 418, 430, 432). Thus in the three monoesters found in latex of *E.triangularis* the hydroxyl esterified with acetic acid in the diesters is free.

In a Craig distribution (n = 5800 transfers) I separates into the mass spectrometrically pure I-460, mixture of I-460/I-472/I-474, mixture of I-472/I-474 and mass spectrometrically pure I-474. The nmr spectrum of I-460 shows a doublet (J = 7 Hz) at 1,15 ppm, characteristic for the geminal methylgroups of an isobutyric acid residue (C_4 -acid). In the nmr spectrum of I-474 a CH_3 -doublet at 1,13 ppm and a CH_3 -triplet at 0,92 ppm is attributed to an α -methylbutyric

[†]) We are greatly indebted to Dr.R.A.Dyer, Department of Agricultural Technical Services, Botanical Research Institute, Pretoria, Republic of South Africa, for supply of latex.

acid residue (C_5 -acid). The nmr spectrum of the mixture I-472/I-474 exhibits the CH_3 -peaks of the α -methylbutyric acid residue as well as the peaks characteristic for a tiglic acid residue ($C=C-CH_3$ 1,82; 2,0; $=CH$ 6,94 ppm). Therefore the C_5 -acid with one $C=C$ -double bond in I-472 is recognized as tiglic acid.



Transesterification of fraction II with $Ba(OH)_2/MeOH$ yields the parent alcohol III. Besides III a second, more polar product IV is found. After chromatographic separation of III and IV and acetylation with Ac_2O/Py a diacetate of III (V, mp. $138^\circ C$, M^+ 432) and a diacetate of IV (VI, resinous, M^+ 432) is obtained.

The spectral data of V are very similar to those of phorbol-acetates (3,4,5):
 ir (KBr) 3400, 1715, (1700), 1628 cm^{-1} ; uv (MeOH) λ_{max} 196, 235, 334 nm
 (ϵ 12300, 5200, 65); cd (0.1% in dioxane; 276-390 nm); $\Delta\epsilon$ 390 0,000,

$\Delta \epsilon_{343} -0,616$, $\Delta \epsilon_{334} -0,583$, $\Delta \epsilon_{292} 0,000$, $\Delta \epsilon_{276} -0,194$. Only two features of the nmr spectrum of V (Chart 1) differ from those of phorbol-12,13,20-triacetate (4,6): one acetylpeak and the doublet at 5,40 ppm (H-12 in phorbol-12,13,20-triacetate) are missing and an additional broad peak at about 2 ppm (H₂-12) is apparent. Consequently the parent alcohol III is the 12-desoxy-phorbol [4,9,13,20-tetrahydroxy-tigliadien-(1,6)-one-(3)] and V the 12-desoxy-phorbol-13,20-diacetate⁺.

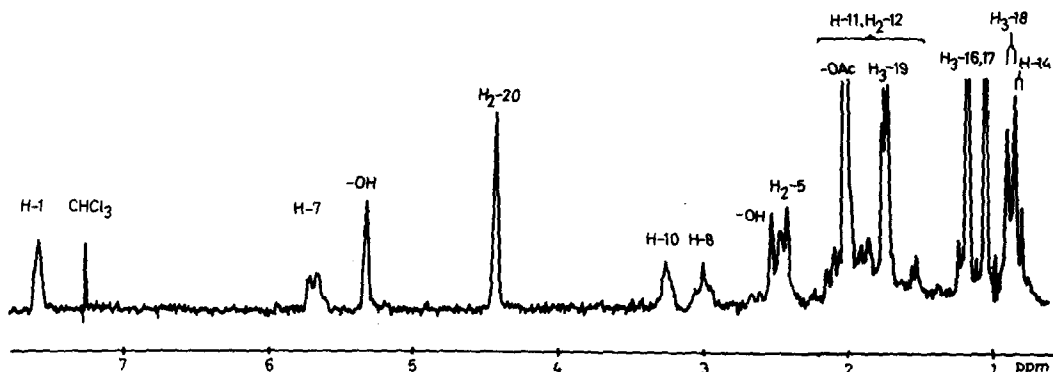


Chart 1: 100 MHz nmr spectrum of 12-desoxy-phorbol-13,20-diacetate (V) in $CDCl_3$ with tetramethylsilane ($\delta = 0,00$ ppm) as internal standard.

The spectral data of VI are very similar to those of 4 α -phorbol-12,13,20-triacetate (8,9): ir (KBr) 3400, 1735, 1718, 1710, 1635 cm^{-1} ; uv (MeOH) λ_{max} 195, 238, 335 nm (ϵ 11300, 5500, 60). As compared to the nmr spectrum of V the nmr spectrum of VI (Chart 2) shows all the characteristic differences which have been found also between phorbol-derivatives and 4 α -phorbol-derivatives (8,9): shift of the peaks of H-1, H-7, H-8 and H-14 to higher field and splitting of the peak of H₂-5 to an AB-system ($\Delta\delta = 1,5$ ppm). Thus VI is the 12-desoxy-4 α -phorbol-13,20-diacetate which is formed by base catalysed epimerization in a way similar to the formation of 4 α -phorbol from phorbol (8,9).

⁺ It is interesting to note that an acetate of 12-desoxy-phorbol was isolated from *Pimelea prostrata* (7).

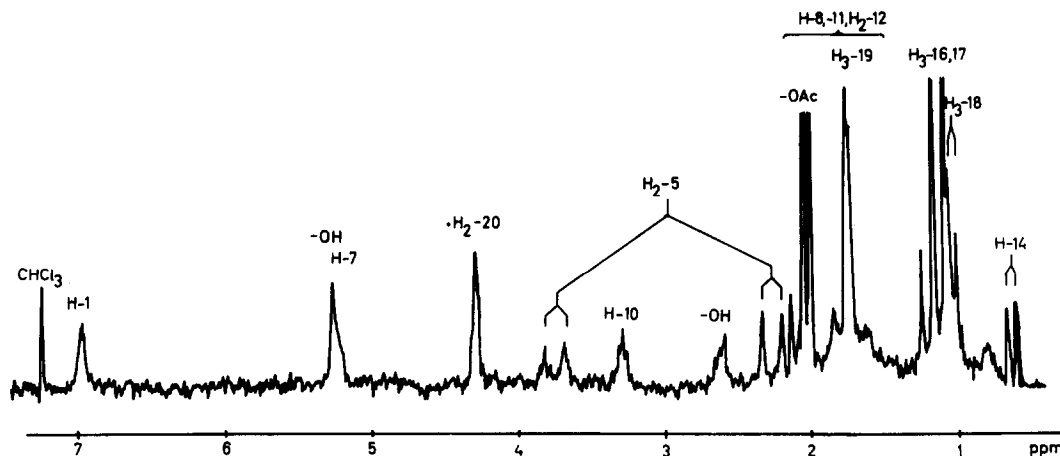


Chart 2: 100 MHz nmr spectrum of 12-desoxy-4 α -phorbol-13,20-diacetate (VI) in CDCl_3 with tetramethylsilane ($\delta = 0,00$ ppm) as internal standard.

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REFERENCES

1. E. Hecker, Verteilungsverfahren im Laboratorium, Verlag Chemie Weinheim/ Bergstraße 1955; Naturwissenschaften **50**, 165, 290 (1963).
2. E. Hecker, Cancer Res. **28**, 2338 (1968).
3. Ch. v. Szczepanski, H. U. Schairer, M. Gschwendt and E. Hecker, Liebigs Ann. Chem. **705**, 199 (1967).
4. E. Hecker, H. Bartsch, H. Bresch, M. Gschwendt, E. Härle, G. Kreibich, H. Kubinyi, H. U. Schairer, Ch. v. Szczepanski and H. W. Thielmann, Tetrahedron Letters (London) **1967**, 3165.
5. M. Gschwendt and E. Hecker, Z. Naturforsch. **23b**, 1584 (1968).
6. E. Hecker, Ch. v. Szczepanski, H. Kubinyi, H. Bresch, E. Härle, H. U. Schairer and H. Bartsch, Z. Naturforsch. **21b**, 1204 (1966).
7. A. R. Cashmore, private communication.
8. E. Hecker, E. Härle, H. U. Schairer, P. Jacobi and W. Hoppe, I. Grassmann, M. Röhrli, H. Abel, Angew. Chem. Intern. Ed. Engl. **7**, 890 (1968); Angew. Chem. **80**, 913 (1968).
9. E. Härle, H. U. Schairer, P. Jacobi and E. Hecker, Liebigs Ann. Chem. in preparation.