STRUCTURE DETERMINATION OF THE NEW TETRACYCLIC DITERPENE INGENOL-TRIACETATE WITH TRIPLE PRODUCT METHODS

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Recently the isolation and chemical characterisation of a new irritant and cocarcinogenic hexadecanoic acid monoester $C_{36}^{H}_{58}^{O}_{6}$ from the latex of Euphorbia ingens and from the seed oil of Euphorbia lathyris was reported (1,2). Mild base



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a $R_2 = R_3 = H;$ $R_1 = CO - (CH_2)_{14} - CH_3$ b $R_1 = R_2 = R_3 = H$ c $R_1 = R_2 = R_3 = COCH_3$

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catalysed transesterification of the resinous ester yields the biologically **inactive resinous** parent diterpene alcohol $C_{20}H_{28}O_5$ - ingenol <u>ib</u> - of unknown constitution - parent ion m/e = 348; ir (KBr): 3410, 1705, 1635 cm⁻¹; uv (MeOH): 6,0 (H-7, d), 5,84 (H-1), 4,32 (H-3 or H-5), 4,2 (H-8) 4,09 (H₂-20) 3,79 (H-5 or H-3), 1,85 (H₃-19), 1,16; 1,1 (H₃-16, H₃-17) 0,98 (H₃-18), 4,4 - 3,2 ppm (4 OH). with acetic acid anhydride/pyridine Ib yields a crystalline triacetate C₂₆H34⁰8, Ic m.p. 195-197⁰, parent ion m/e = 474; ir (KBr): 3430, 1740, 1730, 1705, 1635 cm⁻¹; uv (MeOH): λ_{max} : 194, 292 (ξ_{max} 16800, 230); nmr (CDCl₃): (tetramethylsilane δ = 0,00 ppm) 6,24 (H-7,d), 6,08 (H-1, 5,38 (H-5,s), 4,97 (H-3,s), 4,58, 4,18 (H-20a, H-20b, $J_{AB} = 12,6$ cps), 4,25 (H-8, $J_{H-7/H-8}$ 4,6 cps), 2,22, 2,14, 2,0 (3 CH₃CO), 1,76 (H₂-19), 1,12, 1,09 (H₂-16, H₂-17), 1,0 (H₂-18) 3,24 ppm (1 OH). The constitution and conformation of Ic was determined by X-ray structure analysis (3) using crystals obtained from ether-petroleum ether. Crystallographic data: Space group $P2_12_12_1$, z = 4; $a = 17.58_8^{A}$, $b = 21.55_2^{A}$, $c = 6.75_3^{A}$. The asymmetric unit contains one molecule $(d_{calc}^{1.23} g/cm^3; d_{meas}^{2.2} = 1.22 g/cm^3)$. 2265 reflections were measured with CuKe radiation using a Siemens single crystal diffractometer (0560°, 0/20 scan, 5-point measuring procedure). The structure was determined by the direct method (4,5) which does not require any structural chemical information. The automatic procedure employed is based on a cyclic application of the statistical triple product phase relations (6) and the Sayre equation (7). The free choice of the origin in the unit cell and of either of the two enantiomorphic structures allows the phases of four two-dimensional reflections to be arbitrarily fixed in the space group concerned here. Similarly as in case of the structure analysis of alcohol phorbol (8), two out of the four three-dimensional reflections were used as starting set. The phases of these reflections are continually redetermined during the cyclic procedure. In order to extend the initial phase set, two further two-dimensional reflections were included. A few cycles of the procedure were performed on each of the four resulting phase sets, and the set with the best correlation (according to certain criteria) taken as the correct one. This one alone was then cycled further until the phases of all the unitary structure factors used (the 457 largest ones) were determined. A subsequent U-Fourier synthesis immediately showed 31 out

of 34 atoms of the molecule (excluding hydrogen atoms). A F-Fourier synthesis using all 2265 phases calculated from these positions yielded the remaining three atoms. Using isotropic and anisotropic refinement and difference Fourier synthesis, a final R-factor of 6.2% was obtained. A perspective diagram of the C and O atoms is given in fig. 1a. Fig. 1b shows the positions of the hydrogen atoms in the x,y-projection.



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fig. 1 (a) Perspective diagram of the molecule of ingenol-triacetate (50% probability that the atoms occurs within the ellipsoid). The z-axis is perpendicular to the paper plane and points to the observer.

(b) x,y-projection of a difference Fourier synthesis of ingenol-triacetate showing hydrogen positions. Contours at intervals of 0.1 e/A .

The absolute configuration was determined by measuring the intensity ratios of Bijvoet pairs, for which the calculated anomalous effects were strongest. Chrome radiation was used, the oxygen atoms being the only anomalous scatters (9). The absolute configuration of Ic is that shown in the figures. It corresponds to that of phorbol (II, 8, 10).

The new tetracyclic diterpene skeleton of ingenol <u>ib</u> exhibits a quarternary center interlinking the two seven membered rings with the five membered ring. The plane of the latter occupies a position almost perpendicular to that of the rest of the molecule. As may be seen by comparison of <u>ib</u> with II the tiglian skeleton may be formed following reduction of the carbonyl group in position 9 of <u>ib</u> and subsequent Wagner-Meerwein-rearrangement. The new parent hydrocarbon of <u>ib</u> is proposed to be called in g e n a n. According to spectroscopic and chemical data the new irritant and cocarcinogen $C_{36}H_{58}O_6$ is ingenol-3-hexadecanoate (<u>Ia</u>,11).

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