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STRUCTURE DETERMINATION OF A SOLVATE OF PHORBOL - THE DITERPENE PARENT OF THE TUMOR PROMOTORS FROM CROTON OIL

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Structural analysis of solvates of biologically active compounds may suggest active sites of such molecules for interaction with receptor sites in the cell. Thus the parent alcohol of the most active tumor promotors known so far (1), phorbol, yields a typical solvate with one molecule of ethanol, "alcohol phorbol" m.p. 238-239°C (2), the structure of which was determined by three-dimensional x-ray analysis using crystals obtained from a 10:1 ethanol-water mixture. Crystallographic data: Space group $P2_12_12$, z = 4; a = 18.57_5 Å, b = 12.77_7 Å, c = 9.61_1 Å, d_{meas} = 1.21 g/cm^3. The asymmetric unit contains one molecule of phorbol $C_{26}H_{28}O_6$ and one of ethanol (d_{calc} = 1.20 g/cm^3). 1916 independent reflections were measured with CuKα radiation using a Siemens single crystal diffractometer ($\theta \leq 60^\circ$, $\theta/2\theta$ scan, 5-point measuring procedure).

The position of the 20 carbon and 6 oxygen atoms of the phorbol molecule were determined by a direct procedure (3) using a cyclic application of the statistical phase relationships of the triple product equation (4) and Sayre's equation (5). In this new procedure the origin of the cell is temporarily fixed by assigning arbitrary phases for three very strong three-dimensional reflections.

As the phases of the three starting reflections are continually redetermined during the cyclic procedure the origin moves to one of the eight allowed positions (the three phase shifts were $+40^{o}$, $+82^{o}$, -53^{o}). This removes the previous limitation of the starting reflections to two-dimensional ones. The 334 largest unitary structure factors were used in the phase determination. A U-Fourier synthesis using the phases thus determined yielded all 26 atoms of the phorbol skeleton. Calculation of all structure factors from these positions yielded an R-factor of 31.7%. A final R-factor of 5.4% was reached using difference Fourier syntheses and isotropic and anisotropic refinement. The ethanol molecule occurs in two statistical positions for which the oxygen atoms coincide. The positions appear to have equal occupancy (Fig.1). Fig.2 shows the positions of the 27 (out of 28) hydrogen atoms found.

It is interesting to notify that the alcohol molecule in the crystallized "alcohol phorbol" is attached to hydroxyl group 9 (oxygen O_6, fig.1). In appropriate solutions of the biologically active phorbol-12.13-diesters (1) this same hydroxyl forms a strong intramolecular hydrogen bond with the carbonyl of the ester group in position 13 as revealed by infrared- and NMR-spectroscopy (6). Thus it might be suspected that hydroxyl 9 is one of the essential groups for interactions of phorbol-12.13-diesters with receptor sites in the cell.

Some months ago Crombie, Games and Pointer (7) published a paper on the chemistry of phorbol claiming to report on "a novel tetracyclic diterpene". However, their paper essentially confirms the structure and reactions of phorbol-esters as summarized already by us in 1966/67 (1,8-13) and as reported also by correspondents (14,15). This is especially surprising, as one of the authors (M.L.Games) attended a lecture dealing with phorbol and its chemistry delivered by E.Hecker at the Chester Beatty Institute for Cancer Research in London, January 11, 1967 (see (1) footnote p.282). Unfortunately, the terminology used by these authors (7) differs from the rationalized proposals made by us according to international conventions. In order to avoid confusion in the literature it would be helpful if the authors of the above mentioned paper (7) would adopt the terminology proposed by us (8-13,16).

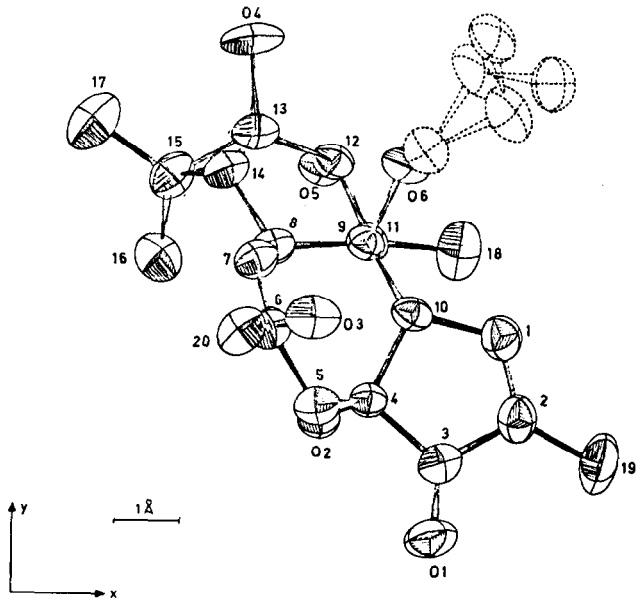

Fig.1: Perspective diagram of the phorbol molecule (50% probability that the atom occurs within the ellipsoid) and the ethanol molecule (dashed) in its two statistical positions (20% probability). The Z-axis points out of the paper.

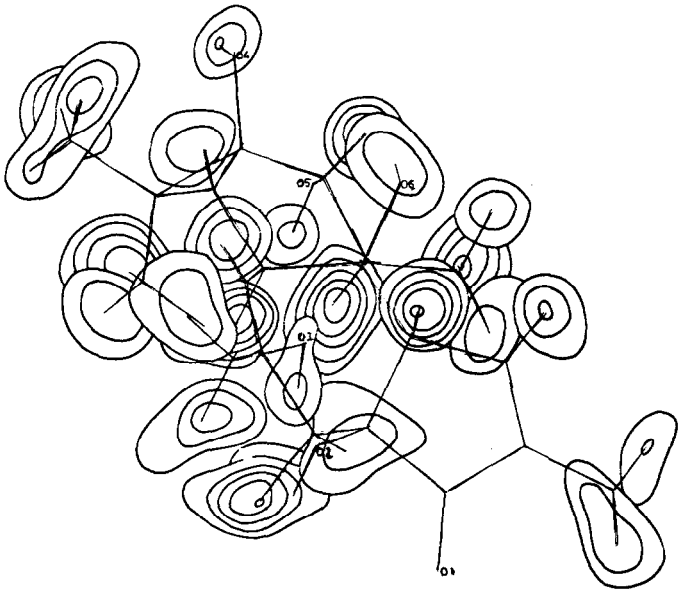

Fig.2: x,y-projection of a difference Fourier synthesis of phorbol showing hydrogen positions. Contours at intervals of 0.1 e/Å^3.

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