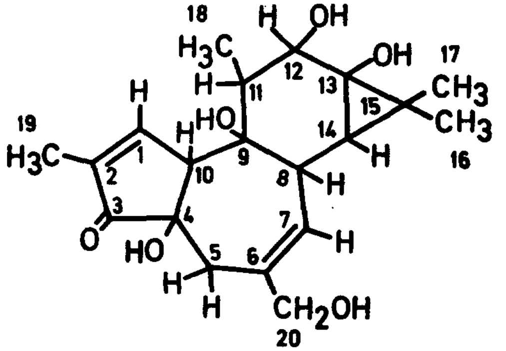
Tetrahedron Letters No.33, pp. 3165-3170, 1967. Pergamon Press Ltd. Printed in Great Britain.

STRUCTURE AND STEREOCHEMISTRY OF THE TETRACYCLIC DITERPENE PHORBOL FROM ÇROTON TIGLIUM L.

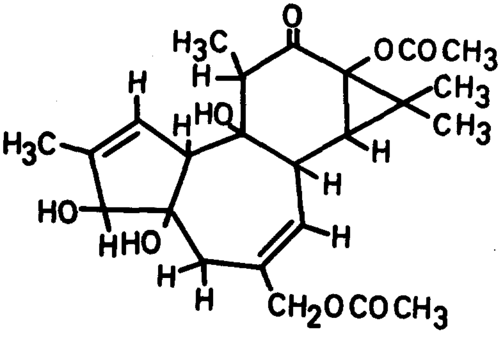
E.Hecker, H.Bartsch, H.Bresch, M.Gschwendt, E.Härle, G.Kreibich,
H.Kubinyi, H.U.Schairer, Ch.v.Szczepanski and H.W.Thielmann
Biochemisches Institut, Deutsches Krebsforschungszentrum, Heidelberg, Germany

(Received 24 May 1967)

A revised structure I for phorbol1,2), the polyfunctional parent alcohol of the tumour promoting compounds from croton oil1-4 and earlier communications) has been proposed on the basis of additional chemical and physical data3,4). As compared to our first proposal5), the partial structures6,7) 10-1-2(19)-3, 4-5-6(20)-7-8-14-13-15 and 12-11(18) remain unchanged. With two structural elements modified they constitute the new perhydro-cyclopropabenz-azulene8) skeleton I.



I



II

Instead of a reducing secondary acyloin group as suggested by Kauffmann et al.9) and accounted for in our first proposal5) phorbol contains a non reducing tertiary acyloin group: the circular dichroism of the two isomeric monoketones phorbol-13,20-diacetate5,7) and resinous neophorbol-13,20-diacetate (448,5) II is additive, giving the circular dichroism of the diketone phorbol-12-on-13,20-diacetate5), thus indicating the non-existence of an α-diketo group in the latter compound. II is obtained by selective reduction of phorbol-12-on-13,20-diacetate with sodium borohydride. Etherification of the hydroxyl at C-4 to give phorbol-12,13,20-triacetate-4-methylether (504,6) changes the sign of the circular dichroitic absorption of the α,β-unsaturated carbonyl chromophore at 360 mμ. During reduction of phorbol-pentaacetate6) with sodium borohydride intramolecular migration of an acetyl group to the newly formed hydroxyl at C-3 takes place. In phorbol-12,13-20-triacetate5,6) the hydroxyl at C-4 is removed by zinc/acetic acid to give 4-desoxy-phorbol-12,13,20-triacetate $C_{26}H_{34}O_8$ (476,6), m.p. 183-85°C. Reduction of phorbol-12,13,20-triacetate with sodium borohydride yields resinous phorbol-3-ol-12,13,20-triacetate (characterized as phorbol-3-ol-3,12,13,20-tetraacetate, $C_{28}H_{38}O_{10}$, m.p. 183-85°C) which may be cleaved by sodium periodate to give 3,4-seco-phorbol-12,13,20-triacetate III $C_{26}H_{34}O_9$, m.p. 124-26°C, the structure of which has been elucidated by nuclear magnetic double resonance measurements after reduction of both carbonyl groups with sodium borohydride3).

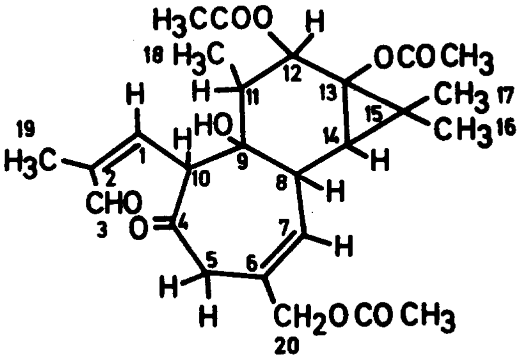
Phorbol definitely contains an α-glycol group with the tertiary cyclopropane hydroxyl4,5,7) and - contrary to our first proposal5) - the secondary hydroxyl participating. The reducing capability of phorbol is a property related to the tertiary cyclopropane hydroxyl (table 1).

TABLE 1

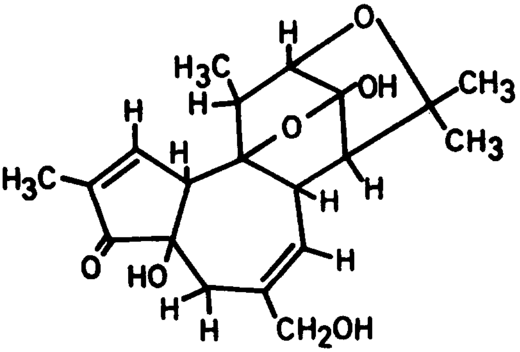
Reducing Activity of Phorbol and some of its Derivatives

Compound	Tollens Reagent	Fehling's Reagent
Phorbol5,6)	+	+
Phorbol-20-trityl-ether	+	+
Phorbol-13,20-dimethyl-ether (392,5)	-	-

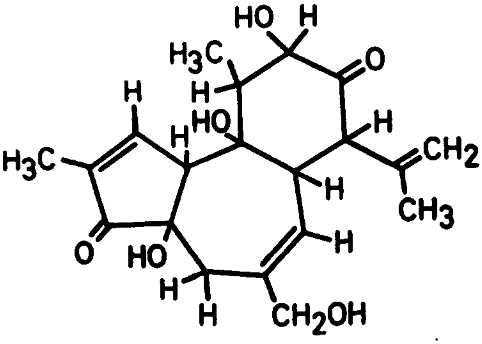
By oxidation of phorbol with 1 mole of lead tetraacetate, generation of acetone, as reported by Kauffmann et al.10), is not observed and only C-20-compounds



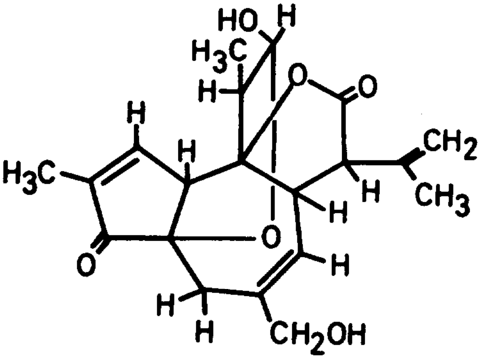
III



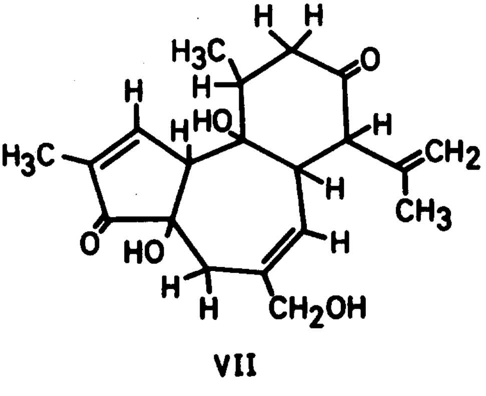
IV



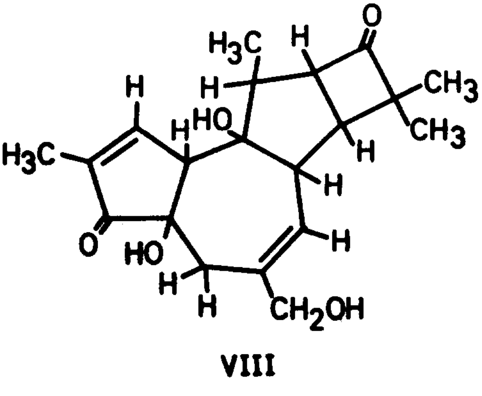
V



VI



VII



VIII

are obtained (overall yield of chromatographically purified compounds 70%). The main product (37%) is tiglophorbol $C_{20}H_{26}O_6$, m.p. 220-23°C +) (diacetate $C_{24}H_{30}O_8$ (446,5), m.p. 98-100°C) which is most probably the ether-semiketale IV. Furthermore bisdehydrophorbol V (25%) $C_{20}H_{26}O_6$ (362,4), decomposing at 220°C, was obtained, yielding a V-12,20-diacetate $C_{24}H_{30}O_8$ (446,5), m.p. 186-88°C, all the hydrogens of which have been identified by nmr measurements. V-12,20-diacetate is also obtained (67%) by oxidation of phorbol-12,20-diacetate with lead tetraacetate. As a minor product (8%) of the lead tetraacetate oxidation of phorbol a compound $C_{20}H_{24}O_6$ (360,4), m.p. 196-98°C, is obtained which is most probably the phorbolacton-semiacetale VI yielding a diacetate $C_{24}H_{28}O_8$ (444,5), m.p. 124-26°C. IV and VI are also generated by oxidation of phorbol with 2 moles of sodium periodate. Bisdehydrophorbol V yields VI with sodium periodate, but V-12,20-diacetate does not react with sodium periodate to give VI-diacetate.

Treatment of phorbol I with 0,02 n sulfuric acid yields crotophorbolon, m.p. 229-30°C, which is - at variance with earlier suggestions[10,11] - a C-20-compound $C_{20}H_{26}O_5$ (346,4). Structure VII is in agreement with all physical and chemical data of crotophorbolon. By reaction of phorbol-13,20-diacetate[5,7] with mesylchloride in pyridine crotophorbolon-enol-13,20-diacetate $C_{24}H_{30}O_7$ (430,5), m.p. 170-71°C, is obtained, and can be converted into VII by base catalysed transesterification. In agreement with structures I and VII respectively, phorbol-12,20-diacetate cannot be converted to VII with $POCl_3$/pyridine. Besides VII as a further product of sulfuric acid treatment of phorbol phorbobutanon VIII $C_{20}H_{26}O_5$ (346,4), m.p. 217-19°C is obtained, the structure of which has been established by nuclear magnetic double resonance measurements. The 20-monoacetate of VIII was previously[3,5] described.

From the characteristics of the compounds mentioned and based on cis-configuration at C-13 and C-14 the relative configuration of 7 of the 8 asymmetric centres of phorbol - referring to H-14 - may be derived as: 4-OH-trans, 8-H-trans, 9-OH-cis, 11-CH_3-cis, 12-OH-trans, 13-OH-cis (IX or mirror image thereof).

[+] Prof.Dr.Th.Kauffmann, Münster, kindly supplied a sample of tiglophorbol for identification by IR spectra.

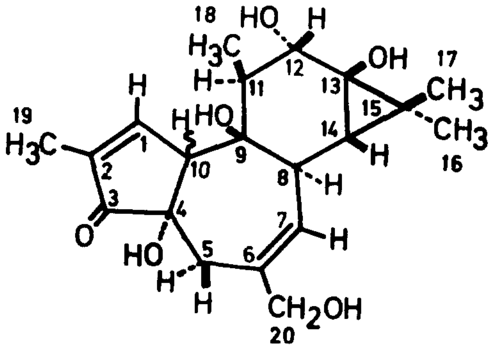
Accounting for the conformational effects of the cyclopropane ring and excluding a trans-diaxial linkage of the six- and seven-membered rings, a half chair conformation of the 6-membered ring of phorbol is in accordance with the relevant coupling constants (table 2). From circular dichroitic measurements and the rule for α,β-cyclopropyl-ketone-chromophores[12,13] the absolute configuration of neophorbol-13,20-diacetate II may be derived with H-14 in β-configuration. Phorbol therefore is a 4,9,12α,13,20-pentahydroxy-tigliadien-(1,6)-on-(3)+) IX. The configuration at C-10 remains to be determined.

The carbon skeleton of tiglian may be split into 4 head to tail linked isoprene units.

TABLE 2

Coupling Constants (in cps) of Phorbol (I) and Bisdehydrophorbol (V) from Nuclear Magnetic Double Resonance

Compound	H-8,14		H-11,12	
	J	conformation	J	conformation
Phorbol	5	trans-8-axial- 14-pseudoequatorial	11	trans-diaxial
Bisdehydrophorbol	13	trans-diaxial	12	trans-diaxial



IX

+) For the saturated parent hydrocarbon with the same configuration at those asymmetric centres occurring also in phorbol the trivial name tiglian is proposed (see loc.cit.5,6).

For compounds whose empirical formulae are given, correct elemental analyses have been obtained. Molecular weights recorded have been confirmed by mass spectrometry. Melting points are uncorrected.

Measurements and stimulating discussions of: mass spectra by Dipl.Chem. C.Wünsche, Heidelberg and Dr.W.Schäfer, München; nmr spectra by Dr.A.Mannschreck, Heidelberg, Dr.J.Jochims, Heidelberg, Dr.A.Melera, Zürich, Dr.U.Scheidegger, Zürich, and Dr.J.Sonnenbichler, München; circular dichroism by Doz.Dr.G.Snatzke, Bonn, and Dipl.Chem.V.Scheidel, Heidelberg, are gratefully acknowledged.

REFERENCES

1. E.Hecker, lectures given at the Marburger Chemische Gesellschaft (May 20, 1966), the Gesellschaft Deutscher Chemiker, Ortsverband Göttingen (June 16, 1966) and the Max-Planck-Institut für Biochemie, München (October 4, 1966).
2. E.Hecker, Naturwissenschaften 54, 282 (1967).
3. H.Bartsch, H.Bresch, M.Gschwendt, E.Härle, G.Kreibich, H.Kubinyi, H.U. Schairer, Ch.v.Szczepanski, H.W.Thielmann and E.Hecker, Z.anal.Chem. 221, 424 (1966).
4. E.Hecker, Molekulare Biologie des malignen Wachstums, 17. Colloquium der Gesellschaft für physiologische Chemie, Springer Verlag, Berlin-Heidelberg-New York 1966, p.105.
5. E.Hecker, H.Kubinyi, Ch.v.Szczepanski, E.Härle and H.Bresch, Tetrahedron Letters 1965, 1837.
6. E.Hecker, Ch.v.Szczepanski, H.Kubinyi, H.Bresch, E.Härle, H.U.Schairer and H.Bartsch, Z.Naturforsch. 21b, 1204 (1966).
7. Ch.v.Szczepanski, H.U.Schairer, M.Gschwendt and E.Hecker, Liebigs Ann. Chem. in press.
8. E.Härle, W.Koch and E.Hecker, Chem.Ber. 100, 795 (1967).
9. Th.Kauffmann and H.Neumann, Chem.Ber. 92, 1715 (1959).
10. Th.Kauffmann, A.Eisinger, W.Jasching and K.Lenhardt, Chem.Ber. 92, 1727 (1959).
11. B.Flaschenträger and F.v.Falkenhausen, Liebigs Ann.Chem. 514, 252 (1934).
12. K.Schaffner and G.Snatzke, Helv.chim.Acta 48, 347 (1965).
13. C.Djerassi, W.Klyne, T.Norin, G.Ohloff and E.Klein, Tetrahedron 21, 163 (1965).