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On the Active Principles of Croton Oil

X.* Preparation of Tritium Labeled Croton Oil Factor A_1 and Other Tritium Labeled Phorbol Derivatives**

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Zusammenfassung. Es werden Verfahren zur spezifischen Tritiummarkierung von tumorpromovierenden Phorbolestern mit hoher biologischer Aktivität sowie der entsprechenden inaktiven Derivate beschrieben. TPA-acetyl-^{3}H (Wirkstoff A_1 aus Crotonöl) wird mit einer spezifischen Aktivität von 6,3–100 mc/mmol erhalten, wenn man in 12-O-Tetradecanoyl-phorbol-20-acetat bzw. in 12-O-Tetradecanoyl-phorbol-20-tritylather die freie Hydroxylgruppe 13 mit Acetathydrid-^{3}H verestert und danach die Schutzgruppe an OH-20 entfernt.

TPA-20-^{3}H (Wirkstoff A_1 aus Crotonöl) der partialsynthetische Tumorpromotor PDD-20-^{3}H und das entsprechende inaktive Epimere 4-α-PDD-20-^{3}H sowie Phorbol-20-^{3}H werden durch selektive Reduktion der entsprechenden 20-Aldehyde mit Natriumborhydrid-^{3}H synthetisiert. Die spezifischen Aktivitäten der markierten Verbindungen liegen zwischen 1,35 und 7,5 C/mmol. Bei der Markierung als Zwischenprodukte anfallende neue Porbolderivate werden beschrieben.

Summary. Procedures are described for specific tritum labeling of some highly active tumor promoting phorbol esters and for corresponding biologically inactive analogues. TPA-acetyl-^{3}H (croton oil factor A_1) with 6.3 – 100 mC/mmole is obtained following acetylation of 12-O-tetradecanoyl-phorbol-20-acetate and 12-O-tetradecanoyl-phorbol-20-tritylether in 13-position with acetic-anhydride-^{3}H and removal of the protecting groups in 20-position.

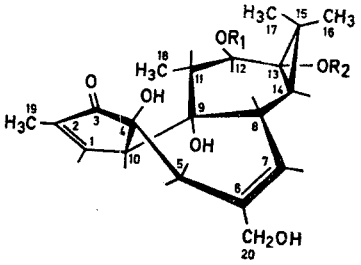
TPA-20-^{3}H (croton oil factor A_1), the tumor promotor PDD-20-^{3}H and the corresponding biologically inactive 4α-epimer 4α-PDD-20-^{3}H as well as phorbol-20-^{3}H are prepared by seletive reduction of appropriate 20-aldehydes with sodium borohydride-^{3}H. The specific activities of the labeled compounds range between 1.35 and 7.5 C/mmole.

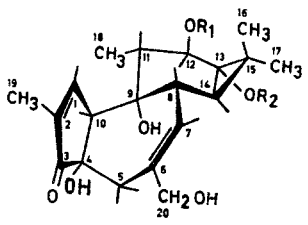
As intermediates of the reaction sequences developed for preparation of the labeled compounds some new derivatives of phorbol are described.

The croton oil factor A_1 (12-O-tetradecanoyl-phorbol-13-acetate, TPA) and phorbol-12,13-didecanoate (PDD, see Fig. 1a) are the most active tumor promotors so far known (Hecker and Bresch, 1965; Hecker 1968; Thielmann and Hecker, 1969). However, the 12-ethylether analogue of TPA (TPE, Fig. 1a) and the 4α-epimer of PDD (4α-PDD, Fig. 1b) have been found to be biologically inactive (Kreibich and Hecker, 1968a; Härle and Hecker, 1970; see also Table 1). For certain biochemical and for autoradiographic investigations biologically active and inactive phorbol derivatives specifically labeled with tritium are desired. In the following the preparation of highly tritium labeled TPA, PDD, 4α-PDD and phorbol as well as the preparation of some new phorbol derivatives will be described in detail. A preliminary account of certain parts of this communication has been given elsewhere (Kreibich and Hecker, 1968b).

* IX. Comm.: Dresch et al., 1968.

** Herrn Professor Dr. K. H. Bauer, Heidelberg, zum 80. Geburtstag gewidmet.





TPA: $R_1 = CO—(CH_2)_{12}—CH_3$, $R_2 = COCH_3$; 4α-PDD: $R_1 = R_2 = CO—(CH_2)_8—CH_3$
PDD: $R_1 = R_2 = CO—(CH_2)_8—CH_3$;
TPE: $R_1 = CO—(CH_2)_{12}—CH_3$, $R_2 = CH_2CH_3$.

Fig. 1a and b. The tumor promotors TPA and PDD and their biologically inactive analogues, TPE and 4α-PDD

Table 1. *Irritant dose 50 (ID_{50}) assayed on the mouse ear (Hecker et al., 1966) and average latency period (t_{50}) assayed on initiated mouse skin* (Hecker, 1966, 1968)

Phorbol derivatives	Irritation ID_{50}[a] [mμM/ear]	Tumor promotion[b] t_{50} [weeks]	dose/appl.[c] p [μM]
TPA	0.016	9.3	0.02
TPE[e]	>41	—[d]	0.02
PDD[f]	0.010	13.5	0.02
4 α-PDD[g]	>37	—[d]	0.02

[a] Standard deviation: 1.3.
[b] 14/14 NMR mice initiator: $i = 0{,}1$ μM 7.12-dimethyl-benz[a]anthracene.
[c] Significance level $\alpha = 0.05$.
[d] No significant regression.
[e] Kreibich and Hecker, 1968a.
[f] Thielmann and Hecker, 1969.
[g] Härle and Hecker, 1970.

Materials and Methods

Mass spectra are recorded at 70eV with the mass spectrometer CEC-21-110 B from Bell & Howell GmbH, Friedberg (Germany). For measuring NMR-spectra (internal standard: Si-$(CH_3)_4$; $\delta = 0.00$ ppm) the spectrograph A-60 from Varian AG Zürich (Swiss) is used. IR-spectra are taken by the grid spectrophotometer 521 from Bodenseewerke Perkin & Elmer & Co. GmbH, Überlingen (Germany). UV-spectra are masured by the UV-spectral photometer DK 2a far UV from Beckman Instruments GmbH, München (Germany). Far UV-spectra are recorded in methanol in a 0,1 cm cuvette while purging with N_2. A solution of cholesterol in methanol (λ_{max} 194 nm; $\varepsilon = 9200-9600$) is used as a standard. $NaBH_4$-^3H and acetic anhydride-^3H with various specific activities are purchased from the Radiochemical Center, Amersham (England). Plates for preparative thin-layer chromatography are prepared with silica gel PF_{254} from E. Merck AG, Darmstadt (Germany). For analytical thin-layer chromatography dust free plates F_{254} from E. Merck AG are used. The spots of the chromatograms are located under UV-light (254 nm) and/or by spraying with vanillin/sulfuric acid reagent (Mathews, 1963) followed by heating to 110 °C. For solvents the chemical formula or the following abbreviations are used: acetone (a), diethylether (e), ethylacetate (ea), benzene (b), cyclohexane (cyclo), petroleum ether (pe). For removal of solvents a rotatory evaporator is used.

Radiochemical purity is checked with the Scanner for thin-layer plates LB 2721 with integration unit from Berthold, Wildbad (Germany). Activities of the labeled phorbol derivatives are determined in the liquid scintillation spectrometer "TriCarb" model 3003, Packard Inc., La Grange (USA); scintillator solution: 15 ml from a solution of 5,5 g Premix M, (Packard) in 1 l of toluene p.a.) and by determination of the UV-extinction based upon the following molar extinction coefficients in ethanol: ($\pi - \pi^*$absorption).

$$\text{TPA } \lambda_{max}\ 230\ \varepsilon_{max}\ 5200;\ \text{TPE } \delta_{max}\ 235\ \varepsilon_{max}\ 5500\,,$$

$$\text{PDD } \lambda_{max}\ 231\ \varepsilon_{max}\ 5500;\ 4\alpha\text{-PDD } \lambda_{max}\ 238\ \varepsilon_{max}\ 7550\,.$$

Counting efficiency and possible quenching is determined by internal and external standardisation.

Starting Material for the Preparation of TPA-acetyl-^3H (see Chart 1).

12-O-tetradecanoyl-phorbol-20-acetate: 1.5 g 12-O-tetradecanoyl-phorbol (Bresch *et al.*, 1968) is dissolved in 18.8 ml of abs. pyridine and cooled to −20 °C, while stirring 0.272 ml of acetic anhydride (1.1 equiv.) is slowly added. The reaction mixture is kept at −20 °C and after 24 hrs it is treated as described earlier (v. Szczepanski *et al.*, 1967). 12-O-tetradecanoyl-phorbol-20-acetate (yield 52%) is obtained as colorless resin after separation by thicklayer chromatography (b : ea = 1 : 2) from starting material from the byproduct 12-O-tetradecanoyl-phorbol-12.20-diacetate and from small amounts of other impurities.

Rf: (b/ea = 1/1) 0,4 .
MS: parent ion m/e = 616 .
NMR ($CDCl_3$) : H_2−20: 4.49 (S); H-12: 4.88 (D); 3 OH (exchangeable) 3.2−1.5 ppm .
IR (CH_2Cl_2) : ν_{OH} : 3560, 3470; ν_{C-O} : 1730, 1700; $\nu_{C=C}$: 1625 cm^{-1} .
UV (CH_3OH) : λ_{max} 194, (227), (245), 329 nm; ε 12310, (5500), (4850), 83 .

Acetylation with "Cold" Acetic Anhydride (see also Chart 1). All flasks have to be dryed over P_2O_5.

In preliminary trials 0.03−0.05 mmole of either 12-O-tetradecanoyl-phorbol-20-acetate or − phorbol-20-tritylether (Bresch *et al.*, 1968) and 3−5 drops of dry pyridine are dissolved in 1 ml of dry benzene containing 0.008−0.025 mmole of cold acetic anhydride. The reaction mixture is kept under nitrogen for 24 hrs until the volatile compounds are removed by lyophilisation. In the same flask the protecting groups are split off according to either one of the following procedures.

Removal of the 20-acetyl Group. The lyophilized residue is dissolved in 5 ml of a 1.5×10^{-2} molar solution of perchloric acid in methanol. After 18 hrs at 20 °C 20 mg of sodium acetate is added. After lyophilization TPA is isolated from the dry residue by preparative thin-layer chromatography with ether (two runs). Yield: between 50 and 60% with respect to the acetic anhydride used.

Removal of the 20-tritylether Group is achieved by treatment of the 20-tritylether for 100 min at 20 °C with 5 ml 5×10^{-4} molar perchloric acid in methanol. The procedure is the same as described for removal of the acetyl group.

If acetic acid is used instead of perchloric acid the lyophilized residue is kept for 10 min in 3 ml of acetic acid at about 100 °C. At a temperature of 75 °C approximately 1 hr is needed to cleave 50% of the tritylether, at 50 °C about 4 hrs and at room temperature three days. The acetic acid is removed by lyophilisation. From the dry residue the compound TPA is isolated by preparative thin-layer chromatography. Yields from 20 "cold" reactions: between 30−45% with respect to the acetic anhydride used.

Acetylations with Acetic Anhydride-^3H (see also Table 3). In the standardized micro set up as described above the "cold" acetic anhydride is substituted by acetic anhydride-^3H. The crude labeled reaction product thus obtained is purified by chromatography in various setems, yields according to Table 3.

Starting Material for the Preparation of Phorbol Derivatives Tritium Labeled at C-20 (see Chart 2). Manganese dioxide (Brauer, 1960) suspended and stirred in methylene chloride is used for oxidation. Because of its low solubility in methylene chloride phorbol-13-acetate is oxidized in acetonitrile. For further details see Table 2. The end of the reaction is determined by thin layer chromatography; if necessary additional manganese dioxide has to be added. The reaction is stopped by filtration of the suspension through a layer of silica gel previously

soaked with water. The silica gel is washed for at least three times with acetone. Then the solution was dried with $MgSO_4$. After removal of the solvent in vacuo slightly yellow resins are obtained which by thin-layer chromatography proved to be pure. Some minor byproducts had to be removed only from the oxidation product of TPE (see remarks table 2).

Table 2. *Reaction conditions as well as starting material and reaction products from the oxidation of phorbol derivatives with manganese dioxide*

starting[a] material	Solvent	Amount of MnO_2 [g]	Reaction time [h]	End product — (yield)	Remarks
0.8 g TPA	200 ml CH_2Cl_2	5	0.8	12-O-tetradecanoyl-20-deoxy-20-oxo-phorbol-13-acetate (88%)	none
0.8 g TPE	133 ml CH_2Cl_2	5.0	0.8	12-O-tetradecanoyl-20-deoxy-20-oxo-phorbol-13-ethylether (40%)	byproducts x) and y)
1.3 g PDD	320 ml CH_2Cl_2	4.6	5	20-deoxy-20-oxo-phorbol-12.13-di-decanoat (50%)	none
1.5 g 4α-PDD	370 ml CH_2Cl_2	5.4	22	20-deoxy-20-oxo-4α-phorbol-12.13-di-decanoate (68%)	stirring in the dark — ness
1.1 g phorbol-13 acetate	250 ml $CH_3C \equiv N$	12	13	20-deoxy-20-oxo-phorbol-13-acetate (55%)	none

[a] Starting materials prepared according to literature quoted in Chart 2.

12-O-tetradecanoyl-20-deoxy-20-oxo-phorbol-13-acetate
Rf: (e) 0,5 .
MS: parent ion m/e = 614.
NMR ($CDCl_3$): H-20: 9.5 (S); H-7: 6.78 (D); 2 OH, 3.45; 5.7 ppm .
IR (CH_2Cl_2): ν_{OH}: 3550, 3390; $\nu_{C=O}$: 1700, 1675; $\nu_{C=C}$: 1630 cm^{-1} .
UV (CH_3OH: λ_{max} 236, 322 nm; ε 17500, 128.

20-deoxy-20-oxo-4α-phorbol-12,13-di-decanoate
Rf: (cyclo/e = 1/10) 0.5 .
MS: parent ion/e = 670 .
NMR ($CDCl_3$): H-20 : 9.4 (S); H-7 : 6.24 (S); 2 OH, 5.1; 2.74 ppm .
IR: (CH_2Cl_2) : ν_{OH} : 3380, 3550; $\nu_{C=O}$: 1705, 1685; $\nu_{C=C}$: 1650 cm^{-1} .
UV (CH_3OH): $_{max}$194, 225, 229 nm; ε 7500, 20200, 117 .

12-O-tetradecanoyl-20-deoxy-20-oxo-phorbol-13-ethylether. The desired product and the two byproducts (x) and (y) were purified by preparative thin-layer chromatography (three runs in the system $CHCl_3$/ae = 5/1.
RF: (e/pe = 4/1), 0.3.
MS: parent ion m/e = 600 .
NMR ($CDCl_3$): H-20 : 9.44 (S); H-7 : 6.76 (D); 2 OH : 3.42, 2.6 ppm.
IR (CH_2Cl_2): ν_{OH} :3550, 3430; $\nu_{C=O}$: 1705, 1680; $\nu_{C=C}$: 1625 cm^{-1} .
UV (methanol): λ_{max}194, 234, 322 nm; ε 8540, 13100, 93 .
Byproducts x and y:
(x): Rf (e/pe = 4/1) 0,5 .
MS: parent ion m/e: 598 .
IR (CH_2Cl_2): ν_{OH} : 3550, 3400; $\nu_{C=O}$: 1710, 1695; $\nu_{C=C}$: 1625, 1605 cm^{-1} .

UV (methanol): δ_{max}195, 296 nm; ε 12123, 4400 .
(y): Rf (e/pe = 4/1) 0.7 .
MS: parent ion m/e = 598 .

20-deoxy-20-oxo-phorbol-13-acetate

Rf: (CH_2Cl_2/a = 3/2) 0.5 .
MS: parent ion m/e = 404 .
NMR (d_5-pyridine): H-20 : 9,64 (S); H-7 : 7,0 (S); 4 OH (exchangeable): 8.2, 6.16, 5.2, 3.3 ppm.
IR (KBr): ν_{OH} : 3600, 3395; $\nu_{C=O}$: 1705, 1660; $\nu_{C=C}$: 1620 cm^{-1} .
UV (methanol): λ_{max}194, 237.5, 324 nm; ε 7150, 16850, 98 .

Reduction of Aldehydes with "Cold" Sodium Borohydride (see also Chart 2). 0.1—0.25 mmole of the appropriate 20-deoxy-20-oxo-phorbol-derivative are dissolved in about 2 ml of abs. methanol. While stirring slowly 0.05—0.125 milliequivalents of "cold" recrystallized sodium borohydride are added from the ampule which is rinsed three times with a final volume of 2—8 ml methanol. After 45 min the reaction is stopped with 0.12—0.4 ml of 10% acetic acid. After another 30 min of stirring the solvents are removed by lyophilization. Excess of starting material and traces of impurities are removed by preparative thin-layer chromatography. Yields of pure product range from 60 to 70%.

Reduction with Sodium Borohydride-^3H. "Hot" runs of the same scale employing sodium borohydride-^{3}H are summarized in Table 4. To obtain radiochemically pure products in some cases rechromatography is necessary. Deviations in the yield as compared to the cold runs may depend on the purity of the labeled starting material obtained commercially.

Phorbol-20-^3H from Phorbol-13-acetate-20-^3H. 0,1 mmole of phorbol-13-acetate-20-^{3}H prepared according to the procedure described above is extracted from thin-layer silica gel and dissolved in 3.8 ml of a 2×10^{-3} molar solution of sodium methoxide in methanol. 10 hours later two drops of acetic acid are added and the dry residue is purified by preparative thin-layer chromatography.

Storage of the Tritium Labeled Phorbol Derivatives. To minimize irradiation decomposition the ^{3}H-labeled compounds are dissolved in acetone (0.5—1.2 mg/ml and stored at —30° C. In case of TPA-20-^{3}H (spec. act. 1.53 c/mmole) five months later only 20% of the material is decomposed whereas in benzene solution under similar conditions 50% decomposition is found within five months. This may be explained by the fact, that crystallizing of the solvent causes high local concentrations of the labeled compound.

Results and Discussion

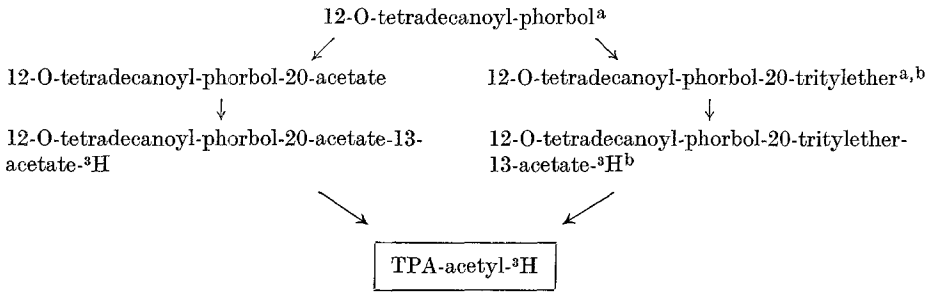
For certain biochemical investigations it is desirable to have two species of one and the same specifically labeled phorbol-12,13-diester: one labeled in one of the acid moieties and the other labeled in the diterpene moiety. To prepare such phorbol derivatives microscale chemical reactions have been developed which allow introduction of the label in one of the last reaction steps to gain the highest possible specific activities with reasonable overall yield.

The use of tritium labeled phorbol esters for biochemical investigations has been mentioned recently by van Duuren 1969. Details as to the preparation of the labeled compound or the localization of the label have not been given.

TPA Labeled in an Acid Moiety

Since acetic anhydride-^{3}H with relatively high specific activity is commercially available it appears efficient to prepare specifically labeled TPA by acetylation of 12-O-tetradecanoyl-phorbol-20-acetate or -20-tritylether (see Chart 1). Both of these products are prepared from 12-O-tetradecanoyl-phorbol described earlier (Bresch *et al.*, 1968) by selective acetylation or tritylation of OH-20 respectively. In each of these products the free OH-13 is acetylated with 0.5 equivalents of acetic anhydride-^{3}H and the protecting groups in 20-position are selectively

Chart 1. *Reaction sequences for preparation of TPA-Acetyl-^3H*
Compounds carrying no literature quotation are being described for the first time



[a] Bresch *et al.*, 1968. — [b] Kreibich and Hecker, 1968b.

removed by acid catalyzed transesterification or cleavage of the ether bond. Thin-layer chromatography of the crude reaction mixture indicates that most of the radioactivity remains at the starting line, whereas little activity is accumulated in the spot of TPA. The chromatograms are also showing a very high radioactivity background and besides labeled TPA byproducts of comparable amount and specific activity are found which did not appear in the "cold" preparations. The data for three different preparations of TPA-acetyl-^3H are summarized in Table 3. The yields and specific activities of the preparations vary considerably depending on the quality of the acetic anhydride-^3H used.

Table 3. *Preparation of TPA-acetyl-^3H*

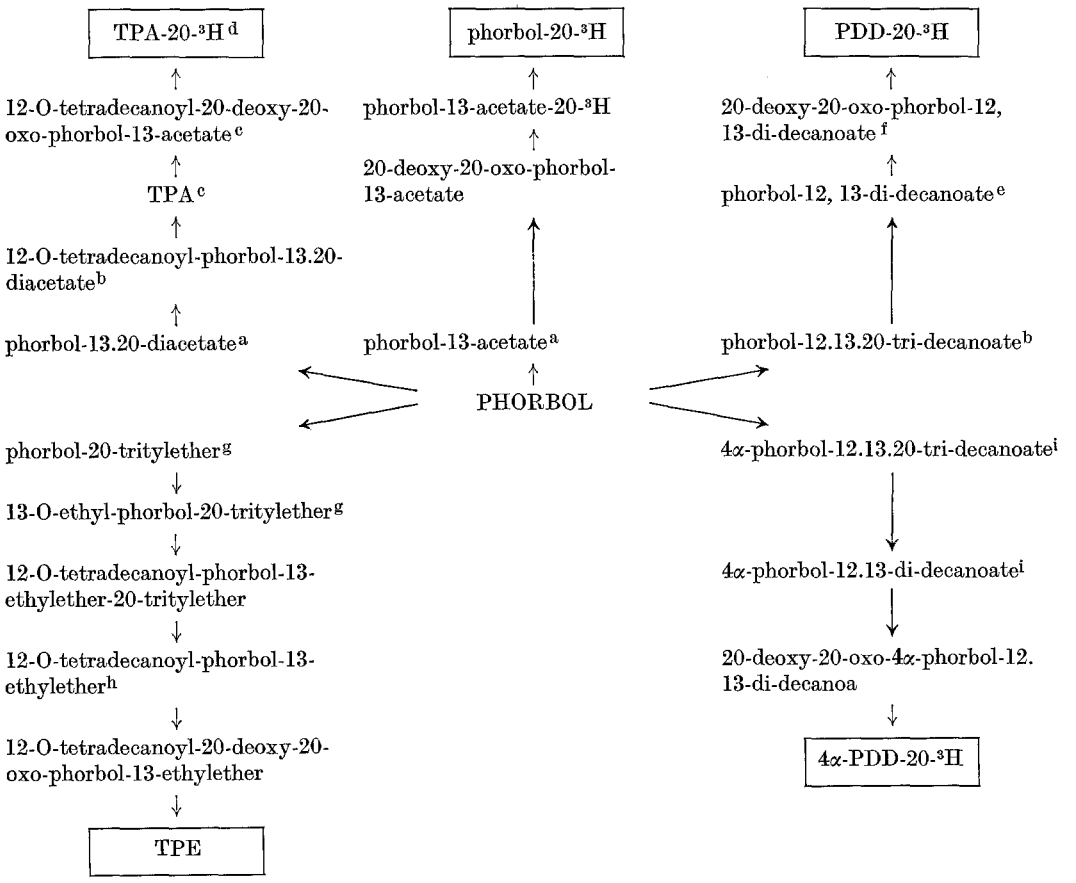
mg	Starting material	Acetic anhydride-^3H		Isolated yield		TPA-acetyl-^3H
		mg	spez. act. mC/mmole	mg	%	spec. act. mC/mmole
26.2	12-O-tetradecanoyl-phorbol-20-tritylether	0.57	4370	0.5	15	6.3
26.2	12-O-tetradecanoyl-phorbol-20-tritylether	0.57	4370	0.7	19	100
60	12-O-tetradecanoyl-phorbol-20-acetate	2.5	4080	0.33	2	36

Phorbol-20-^3H and Phorbol Derivatives Labeled in the Diterpene Moiety

For specific tritium labeling of phorbol and its derivatives in the diterpene moiety the catalytic saturation of double bonds in the parent alcohol (see Fig. 1) was considered. However, by catalytic hydrogenation of biologically active phorbol derivatives not only the double bonds are being saturated but also hydrogenolysis of the allylic hydroxyl in 20-position is observed (Hecker and Bresch, 1965; Bartsch and Hecker, 1968). Either one of these modifications of the parent alcohol results in practically complete loss of biological activity (Borchert, 1968; Schmidt and Hecker, 1970). Thus labeling by catalytic hydrogenation is not feasible.

Selective reduction of the appropriate 20-aldehydes with complex hydrides seems to be more suitable. Such aldehydes have been prepared from croton oil factors A_1, B_1 and B_2 (Hecker, and Bresch, 1965; Hecker and Kubinyi, 1965), from phorbol-12,13-diacetate (Schairer *et al.*, 1968) and from phorbol-12,13-di-decanoate (Borchert, 1968) by selective oxidation with manganese dioxide in methylene-chloride. Accordingly, the 20-aldehydes of TPA, TPE, PDD, 4α-PDD and phorbol-13-acetate are prepared (see Chart 2, Table 2).

Chart 2. *Reaction sequences for the preparation of the tumorpromotors TPA and PDD, their biologically inactive analogues TPE and 4α-PDD and of phorbol labeled with tritium in 20-position* Compounds carrying no literature quotation are being described for the first time



[a] v. Szczepanski *et al.*, 1967. — [b] Bresch *et al.*, 1968. — [c] Hecker and Bresch, 1965. — [d] Kreibich and Hecker, 1968b. — [e] Thielmann and Hecker, 1969. [f] Borchert, 1968. — [g] Kreibich, 1968c. — [h] Kreibich and Hecker, 1968a. — [i] Härle and Hecker, 1970.

Because phorbol is practically insoluble in methylenechloride and because the free cyclopropanol group is easily oxidized (Kreibich and Hecker, 1968c) direct oxidation of phorbol to yield the 20-aldehyd is not feasible. Also phorbol-13-acetate with protected cyclopropanol group is practically insoluble in methylene

chloride. However, it may be oxidized with reasonable yields in acetonitrile (see Table 2).

The conditions for selective reduction of the 20-aldehydes in the milligram scale with sodium borohydride-^{3}H are thoroughly investigated with the 20-aldehyde from phorbol-12,13-diacetate as a model compound. With pyridine, tetrahydrofurane, dioxane, ethylacetate and diglyme as solvents the selectivity of the reduction is not satisfactory. Increasing selectivity can be observed if i-propanol, ethanol or methanol are used as solvents. High selectivity is obtained if the 20-aldehydes are reduced in methanol at 0° C with sodium borohydride in an equivalent ratio of 2 : 1. Also, successful reactions require extremely pure sodium borohydride which is obtained by recrystallization from diglyme (Brown *et al.*, 1955).

Table 4. *Tritium labeled derivatives of phorbol and 4α-phorbol as obtained by reduction of the corresponding 20-aldehydes with sodium borohydride-^{3}H*

Phorbol-derivatives labeled at C-20	Preparative thinlayer chromatography in the system	Spec. act. mC/mmole	Yield in relation to the starting material $NaBH_4$-^{3}H	
			mg	%
TPA-20-^{3}H	1 × e[a]	1530	19	68
		7500	60	93
PDD-20-^{3}H	1 × e/cyclo = 10/1	7250	19.5	25
4α-PDD-20-^{3}H	1 × e/cyclo = 10/1	1320	24	47
phorbol-13-acetate-20-^{3}H	1 × ea[a]	1350	15.6	60
phorbol-20-^{3}H	2 × a/ea = 1/1[a]	1350	13	49

[a] Equilibrated atmosphere.

The tritium labeled derivatives of phorbol and 4α-phorbol as obtained by reduction of the corresponding 20-aldehydes with sodium borohydride-^{3}H are recorded in Table 4. 20-labeled phorbol-13-acetate is converted to phorbol-20-^{3}H by base catalyzed transesterification (v. Szczepanski *et al.*, 1967). In contrast to the preparation of TPA-acetyl-^{3}H the reduction of 20-deoxy-20-oxo-phorbol-derivatives with $NaBH_4$ yields only few byproducts. The thin-layer chromatogram of the crude material shows 70—80% of the entire radioactivity in the spot of the reduction product. Less than 5% of the radioactivity is detectable as a small peak with lower Rf-value corresponding to the 3-deoxy-3-hydroxy compound. Only little radioactivity is found at the starting line and at the front of the chromatogram. Using the most active preparations of sodium borohydride-^{3}H presently available the specific activities of the phorbol derivatives labeled in the diterpene moiety range within roughly 10-100 times the specific activities of TPA-acetyl-^{3}H (see Table 3).

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References

Bartsch, H., Snatzke, G., Hecker, E.: Zur Chemie des Phorbols, VI Funktionelle Derivate des Phorbol-12-ons und deren Circulardichroismus. Z. Naturforsch. **23b**, 1453—1460 (1968).

Brochert, P.: Über Desoxyphorbole. Diplom-Arbeit Universität Heidelberg 1968.
Brauer, G.: Handbuch der präparativen anorganischen Chemie. S, 672. Stuttgart: F. Enke 1960.
Bresch, H., Kreibich, G., Kubinyi, H., Schairer, H. U., Thielmann, H. W., Hecker, E.: Über die Wirkstoffe des Crotonöls, IX Partialsynthese von Wirkstoffen des Crotonöls. Z. Naturforsch. **23**b, 538–546 (1968).
Brown, H. C., Mead, E., Subba Rao, B.: A Study of Solvents for Sodium Borohydride and the Effect of Solvent and the Metal Ion on Borohydride Reductions. J. Amer. chem. Soc. **77**, 6209 (1955).
Van Duuren, B. L.: Tumor-promoting agents in two-stage carcinogenesis. Int. Symposion on Carcinogenesis and Carcinogen Testing, Boston, Mass., Nov. 8–9, 1967, Progress in exp. Tumor Res. Vol. 11, p. 31–68. Basel-New York: S. Karger 1969.
Härle, E., Hecker, E.: in preparation, 1970.
Hecker, E.: Die cocarcinogene Wirkung der Phorbolester, 17. Coll. der Ges. Physiol Chem. S. 105–116. Berlin-Heidelberg-New York: Springer 1966.
– Cocarcinogenic Principles from the Seed Oil of Croton Tiglium and from other Euphorbiaceae. Cancer Res. **28**, 2338–2349 (1968).
– Bresch, H.: Über die Wirkstoffe des Crotonöls, III Reindarstellung und Charakterisierung eines toxischen, entzündlichen und cocarcinogenen hochaktiven Wirkstoffs. Z. Naturforsch. **20**b, 216–226 (1965).
– Immich, H., Schairer, H. U.: Über die Wirkstoffe des Crotonöls. VI. Entzündungsteste am Mäuseohr. Z. Krebsforsch. **68**, 366–374 (1966).
– Kubinyi, H.: Über die Wirkstoffe des Crotonöls, IV, Reindarstellung und Charakterisierung der entzündlichen und cocarcinogenen Wirkstoffe B_1 und B_2. Z. Krebsforsch. **67**, 176 (1965).
Kreibich, G., Hecker, E.: Über die Verätherung des Phorbols, Vortrag am 2. Heidelberger Symposion über aktuelle Probleme aus dem Gebiet der Cancerologie, S. 155–161. Berlin-Heidelberg-New York: Springer 1968a.
– – ^{3}H-Markierung des cocarcinogenen Wirkstoffes A_1 aus Crotonöl. Referat bei der 6. Jahrestagung der Gesellschaft für Nuklearmedizin, 26.–28. Sept., Wiesbaden, 1968b.
– – Zur Chemie des Phorbols, V. Über einige Äther des Phorbols. Z. Naturforsch. **23**b, 1444–1452 (1968c).
Mathews, J. S.: Steroids, CCXXIII. Color Reagent for Steroids in Thin-Layer Chromatography. Biochim. biophysica Acta (Amst.) **69**, 163–165 (1963).
Schairer, H. U., Thielmann, H. W., Gschwendt, M., Kreibich, G., Schmidt, R., Hecker, E.: Zur Chemie des Phorbols, IV. Polybenzoate und -acetate des Phorbols und Phorbol-3-ols und funktionelle Derivate der Allylgruppierung des Phorbols. Z. Naturforsch. **23**b, 1430 bis 1443 (1968).
Schmidt, R., Hecker, E.: in preparation, 1970.
v. Szczepanski, Ch., Schairer, H. U., Gschwendt, M., Hecker, E.: Zur Chemie des Phorbols, III. Mono- und Diacetate des Phorbols. Liebig Ann. Chem. **705**, 199–210 (1967).
Thielmann, H. W., Hecker, E.: Beziehungen zwischen der Struktur von Phorbolderivaten und ihren entzündlichen und tumorpromovierenden Eigenschaften. Bericht über die 10. wissenschaftliche Tagung des Deutschen Zentralausschusses für Krebsbekämpfung und Krebsforschung e.V., Berlin 1968. Fortschritte der Krebsforschung – Molekularbiologisches Wachstum, Klinik, S. 171–179. Stuttgart-New York: F. K. Schattauer 1969.

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