the lipids could be eliminated, i.e. about 97% of the inactive material of the original oil. Thin-layer chromatography (silicagel, ethylacetate-chloroform = 1:2) of the lower phase produced 9 distinct spots, representing the colourless oil (Rf = 0.9) and the active agents A (Rf = 0.25) and B (Rf = 0.35), mainly.

Chromatography: applying 12 g of the active resin obtained by evaporating the lower phase of the O'Keeffe distribution to the column, the active principles were found in fractions (100 ml each) 235–300 (B, 29% of the resin) and 345–405 (A, 16%). These components migrate as single spots in thin-layer chromatography[1].

Craig distribution: group A separated into the pure compounds A_1 (0.45% of the original croton oil) and A_3 (0.15%), group B into B_1 (0.4%) and B_2 (0.8%). The diagrams shown in Figures 2 and 3 are asymmetric, because the partition coefficients are dependent upon the concentration (Figure 4). This behaviour was most pronounced with compound B_2. Consequently, the countercurrent distribution of B_2 resulted in the most asymmetric diagram. The effect decreases from B_2–B_1, from B_1–A_1, and from A_1–A_3, respectively, as may be concluded from Figures 2 and 3 as well as from Figure 4.

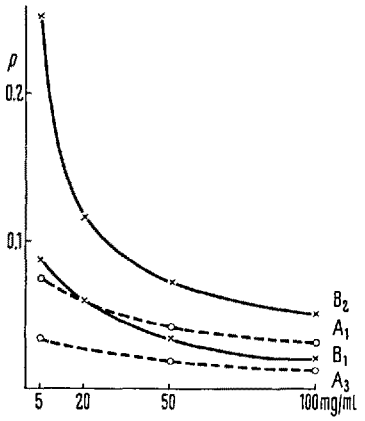

Fig. 4. Partition coefficients (ordinate) of the isolated compounds, determined in the carbontetrachloride-system. They show marked dependence on the concentration (abszissa) of the compounds.

Chemical characterization. IR-absorption spectra of the compounds isolated revealed hydroxylbonds, carbonyl- and esterbonds, and olefinic C=C absorptionbands. Hydrolysis liberated the following fatty acids: from A_1 ($C_{36}H_{56}O_8$) myristic and acetic acid[10], from A_3 ($C_{38}H_{60}O_8$) palmitic and acetic acid[2], from B_1 ($C_{37}H_{58}O_8$) lauric acid and 2-methyl-butanoic acid and from B_2 ($C_{35}H_{54}O_8$) capric and 2-methyl-butanoic acid[11]. All these compounds were shown to be diesters of the polyalcohol phorbol, $C_{20}H_{28}O_6$, analysed in 1934 by FLASCHENTRÄGER[12]. As phorbolesters are known to undergo alkaline hydrolysis easily[12,13] it may be of importance that the simplified procedure no longer uses the elimination of free fatty acids by $2N$ alkalicarbonate, pH = 10.95[1,2,13,14]. It is not yet experimentally excluded that this treatment may cause saponification and re-esterification[12], possibly thus leading to artifacts.

Zusammenfassung. Die schonende Reindarstellung von 4 kokarzinogenen Phorbolestern aus Krotonöl gelingt bereits nach 3 konsekutiven Trennoperationen (O'Keeffe-Gegenstromverteilung, Adsorptionschromatographie, Craig-Verteilung), deren Ausführung präzis beschrieben wird.

J. G. MEYER-BERTENRATH[15]

Medizinische Poliklinik der Universität, 355 Marburg (Germany), 28 August 1968.

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