strated in Figure 3, where the dried C-gamma gels are seen to contain the hexagonal platelets and prisms of the trihydrate Gibbsite and the triangular or hour-glass shaped somatoids of the trihydrate Bayerite[10]. In addition, in these samples, a fine dispersion of background particles (arrow, Fig. 3*A*), is observed with diameters about 200 A.U.

These results show that the particles which constitute the solid phase of SCHMIDT's gels are different from those of the WILLSTÄTTER's C-gamma gels, but are similar to those of the C-beta gels, which are also composed of fibrils, having a Boehmite structure, shown in a previous paper[11], formed by condensation polymerization of the amorphous aluminum hydroxide molecules of the C-alpha gel[13]. Their X-ray diffraction data do not coincide exactly with the data of well crystallized Boehmite because the fibrils are exceedingly small and friable and are neither completely polymerized nor oriented[14]. After autoclaving, SCHMIDT's gels do not change spontaneously into Bayerite and Gibbsite which constitute the C-gamma gels, and from this point of view they are different from the C-beta gel[11]. This stability of crystalline structure, of particle size, and of shape in SCHMIDT's gels make them superior to WILLSTÄTTER's C-beta or C-gamma gels for preparation of adsorbents. In addition to the ammonium alum, other aluminum salts, like the chloride, the nitrate, or the acetate, can be used for preparing gels composed of Boehmite fibrils[12], but the fibrils in these are thicker in diameter[13] and have therefore a small surface area than those from SCHMIDT's gel.

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Résumé

Les auteurs démontrent que le gel d'hydroxyde d'aluminium (gel de SCHMIDT) soumis aux rayons X diffractés et examiné au microscope électronique se révèle formé de particules différant par leur structure, leur morphologie et leur dimensions de celles du gel C-gamma de WILLSTÄTTER, mais semblables à celles du gel C-béta du même nom.

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Crystalline Acetates from 'Croton Resin'

The local irritant and laxative actions of Croton oil (ex. *Croton tiglium*) have been known for a very long time, and to these effects have later been added those of cocarcinogenic action[1], leucocyte migration promotion[2] and 'cord factor'[3] activity. The teams led by CHERBULIEZ[4] and FLASCHENTRÄGER[5] carried out separations some 25 years ago, and further separations have been recently attempted chromatographically[6].

Prompted by the results of MEIER and SCHÄR[2] the oil was worked up as described by CHERBULIEZ for the preparation of the 'Croton resin' ('principe vésicant'), in our case using a continuous countercurrent distribution apparatus[7] for partition between heptane and methanol. This resin was now subjected to chromatography on alumina using eluents graded from benzene through ether-chloroform-methanol, thus yielding about 80 non-crystalline fractions, all with very similar I.R. spectra, and of which the most active in the leucocyte migration test were eluted with benzene-ether (1 : 5) and fitted closely with CHERBULIEZ's description of the original 'principe vésicant'.

[Found for this fraction (3 determinations):
C 68·98; H 8·81; $(CO)CH_3$ 4·73%; M.W. 608; 688[8]; $[\alpha]_D^{20°}$: + 56 ($c = 1·0$ in $CHCl_3$)].

Calculated for $C_{37}H_{56}O_9$:
C 68·91; H 8·75; CH_3 (one) 2·33%; M.W. 644·8

Hydrolysis of the 'Croton resin' using methanolic barium hydroxide[9] or a strongly basic ion-exchange resin (Amberlite IRA-120 in basic form) and subsequent working up by partition between ether and water gave from the aqueous phase a resin that, after crystallization from ethanol, yielded a small amount of crystalline material (m.p. 240–245°, decomp.) of formula $C_{21}H_{32}O_7$.

[Found (8 determinations):
C 64·05; H 8.20%;
(3 determinations):
O 28·66; $(C)CH_3$ 10·44; $(CO)CH_3$ 2·81; $(C)CH_3$ 10·71%.
$C_{21}H_{32}O_7$ requires C 63·61; H 8·14; O 28·25; CH_3 3·78%.]
This was probably identical with the 'Alkohol Phorbol' of FLASCHENTRÄGER[9].

Acetylation in pyridine of the crude water-soluble fraction from the hydrolysis and chromatography on magnesolcelite[10] or alumina gave three crystalline acetates (*A*, *B*, and *C*).

The separation was followed by paper chromatography of alternate fractions (Zaffaroni system formamide-cyclohexane-benzene) and the acetates were recrystallized to paper chromatographic purity from ether or ether-petroleum. The properties of the acetates are summarized in the table. The results from acetate determinations (hydrolysis) were not satisfactory. Further acetylation of

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Properties of the acetates from Croton oil

	From column		m. p.	$[\alpha]_D^{20°}$ 1% in ethanol	Analysis		R_f (Zaffaroni)	I. R. abs. in CH_2Cl_2	U. V. abs. in ethanol
	fraction	eluted with			found %	calculated %			
Acetate A ($C_{23}H_{30}O_8$)	1–10	Benzene	198–200° or 178°	−138°	C 63·29 H 6·86 O 29·40 (C)CH_3 15·5 (CO)CH_3 13·6 M. W. 389	63·58 6·96 29·46 3·45 (one) 434·5	0·94	OH at 2·96 μ C=O at 5·76 μ 5·81 μ	233 mμ (ε = 8,000) 324 mμ (ε = 53)
Acetate B ($C_{21}H_{28}O_7$)	12–16	Benzene-20% ether	118–120°	+ 69°	C 64·13 H 7·38 O 28·22 (C)CH_3 15·6 (CO)CH_3 9·3 M. W. 360	64·27 7·19 28·54 3·82 (one) 392·4	0·65	OH at 2·82 μ 2·95 μ C=O at 5·76 μ 5·85 μ	233 mμ (ε = 4,000) 330 mμ (ε = 51)
Acetate C ($C_{21}H_{28}O_7$)	22–36	Ether-50 % $CHCl_3$ to pure $CHCl_3$	119–121°	− 65°	C 63·98 H 7·35 O 28·76 (C)CH_3 15·9 (CO)CH_3 9·7 M. W. 324	64·27 7·19 28·54 3·82 (one) 392·4	0·41	OH at 2·82 μ 2·95 μ C=O at 5·76 μ 5·83 μ	238 mμ (ε = 5,400) 338 mμ (ε = 49)

acetates *B* or *C* led to acetate *A* exclusively. None of the acetates reacted with 3,5-dinitrophenylhydrazine, and the Legal test was negative, but acetate *B* (possessing weak activity in the leucocyte migration test) reduced Fehling's solution, and acetate *A* exhibited soda fluorescence on paper[11]. It is assumed that in 'phorbol' an additional molecule of ethanol is present (though this cannot be removed on drying) leading to a formula of $C_{19}H_{26}O_6$ for the skeleton of the molecule. It will be noticed that a free OH group is still present in acetate *A*, as indeed there is in the original resin (I. R. absorption at 2·93 μ).

In the croton resin contained in the naturally occurring oil, the polyhydroxy-compound from which these acetates are derived is esterified by saturated fatty acids from C_{10}–C_{16} (even) and acetic acid, but not by C_4–C_8 acids. This was shown by converting the esterifying acids to the corresponding hydroxamic acids by treatment of the resin itself with hydroxylamine and potassium hydroxide, and chromatography on paper by two different methods[12]. It thus seems that the polyester called 'Croton resin' contains three esterified OH groups, two by acetic acid and one by an acid containing 10–16 carbon atoms, one of the acetates being much more difficult to hydrolyze. There is also a free hydroxyl group that is not readily esterifiable, leaving only two oxygen functions in the resin and one in the saponified skeleton unaccounted for.

The biological testing was carried out by Dr. B. Schär in the CIBA (Basle), biological department (Direction Prof. R. Meier). Thanks are extended to Dr. R. Neher and Mr. E. von Arx for assistance with the paper chromatography. Analyses were carried out under the direction of Dr. H. Gysel.

Noted in proof: It has recently been reported that a tumor-promoting principle containing nitrogen has been isolated from croton oil (W. Lijinsky, 375th Meeting of the Biochemical Society, Sheffield, 1958).

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Zusammenfassung

Durch Hydrolyse des aus Krotonöl gewonnenen Krotonharzes wurde eine empfindliche, wasserlösliche Polyhydroxyverbindung gewonnen, die teilweise als das bereits bekannte Alkohol-Phorbol abgeschieden werden konnte. Von ihren 4 Hydroxylgruppen scheinen im Krotonharz 2 mit Essigsäure verestert zu sein. Nur eine der Acetylgruppen ist verseifbar. Die dritte Hydroxylgruppe ist mit einer höheren Fettsäure von 10–16 C-Atomen verestert, die vierte scheint frei vorzuliegen. Zwei der Hydroxylgruppen im Verseifungsprodukt sind acetylierbar. Im Grundskelett der Formel $C_{19}H_{26}O_6$ ist daher eine Sauerstoff-Funktion noch ungeklärt.

A New Evidence for Induction of Respiration Deficiency in Yeast by Acriflavine

Various chemicals have been known to increase the frequency of respiration-deficient variants in yeast. To prove the actual induction by the drug, one must successfully exclude the possibility that the variant cells contained in the inoculum, or spontaneously occurring during growth, might have selective advantage over the normal (respiration-sufficient) cells in a population growing in the presence of the drug. This critical examination was achieved by: (1) Following the time course of population changes and finding the accumulation of variant cells at a rate faster than that of the variant culture alone, as performed by Ephrussi, L'Heritier and Hottinguer[1] on acriflavine; (2) placing a normal mother cell in a medium containing the drug, detaching the buds formed in the presence of the drug by micromanipulator, transferring them separately to drug-free medium, and finding variants to be the majority of thus detached daughter cells, with the mother cell still remaining normal, as demonstrated by Ephrussi and Hottinguer[2] on euflavine

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