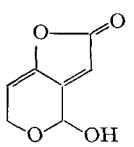
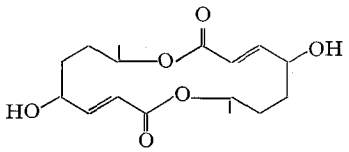
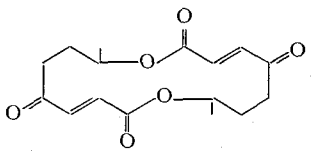
$C_{16}H_{20}O_6$, das mit authentischem Pyrenophorin (III)[4], einem Metaboliten von *Pyrenophora avenae*[5] und *Stemphylum radicinum*[6] identifiziert werden konnte[7]. Diese Daten und Reaktionen lassen für Substanz B, die wir Pyrenophorol nennen wollen, die Strukturformel II ableiten.



I (=A)



II (=B, Pyrenophorol)



III (=Pyrenophorin)

Pyrenophorol (II) zeigte im Gegensatz zum Pyrenophorin (III) im Agardiffusions-Test keine antifungischen Aktivitäten[1].

Summary. The isolation of patulin I and pyrenophorol II from submerse cultures of *Byssochlamys nivea* Westling is described.

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[7] Wir sind Prof. S. Nozoe, Dr. K. Ishibashi und Dr. J. F. Grove für die Überlassung von Proben und Spektren von Pyrenophorin zu tiefstem Dank verpflichtet.

The Structure of Prosapogenin from Quillaja Saponin

The bark of *Quillaja saponaria* Mol. contains a saponin which has been studied for a long time[1].

When submitted to hydrolysis with 1.5 *N* sulfuric acid at 110°, the saponin yielded a product[2,3] which was further studied by Windaus et al.[4] It was named prosapogenin, and was found to be a dibasic acid which contained the original aglycon condensed with an hexuronic acid, and assigned the formula $C_{35}H_{56}O_{12}$. Under other hydrolytic conditions, Windaus could isolate the free aglycon which was afterwards named quillaic acid by Elliott et al.[5], who determined the correct formula $C_{30}H_{46}O_5$. In the early work[2], D-galactose was the only other product identified by hydrolysis.

In the course of a study of the saponin from *Q. saponaria*, the prosapogenin was prepared again by the method of Windaus. After several recrystallizations from ethanol 85%, it was obtained as fine needles, m.p. 219–221° dec.; $[\alpha]_D^{20}$ +16° (CH_3OH); $[\alpha]_D^{17}$ +15.3° (pyr); $[\alpha]_D^{18}$ +18.1° (0.05 *N* NaOH) (no mutarotation) (Lit. m.p. 206–207°[4]) which gave analytical values expected for a glucuronoside of quillaic acid. Found: C, 65.31; H, 8.61. $C_{36}H_{54}O_{11}$ requires: C, 65.23; H, 8.21%.

Hydrolysis of the prosapogenin with 90% formic acid (5 h, 100°, sealed tube) gave a crude product which after evaporation of the formic acid was separated into a water-soluble and a water-insoluble fraction. The water solution showed on paper and thin layer chromatography[6] that it contained glucuronolactone. It was purified through a column of cellulose and the solid residue obtained on evaporation, condensed with *p*-nitroaniline to give crystalline 1-*p*-nitrophenylamino-1-deoxy-D-glucofuranurono-γ-lactone m.p. 133–135° (ethanol 96%); $[\alpha]_D^{20}$ +261° (pyr); ν_{max} 1770 cm^{-1} (γ-lactone) identical (mixed m.p.; I.R.; t.l.c.), to a sample prepared from authentic D-glucuronolactone[7].

The isolation of quillaic acid as methyl ester, from the prosapogenin, was carried out by hydrolysis with 0.6 *N* sulfuric acid at 140–145° (sealed tube). The dried crude insoluble aglycon when treated with diazomethane yielded the methyl ester of quillaic acid which was purified by chromatography on alumina and recrystallized from methanol 90%; m.p. 216–220°; $[\alpha]_D^{20}$ +38.5° (pyr) identical (mixed m.p.; I.R.; t.l.c.) with an authentic sample.

The glucuronic acid is present in the pyranoid form, because on sodium periodate oxidation, 5 moles of the reagent were consumed[8,9]. That the prosapogenin is a β-glycoside was shown by its hydrolysis by the action of β-glucuronidase[10]; the D-glucuronic acid produced was identified by paper chromatography. The β-glycosidic configuration was confirmed by application of Klyne's rule[11]. The calculated value $[M]_{D\ prosap.} - [M]_{D\ aglyc.}$ for the prosapogenin is −171° much nearer to $[M]_D$ −115°, the value of methyl-β-D-glucopyranuronic acid than to the value $[M]_D$ +268° of the α-anomer[12]. The $[M]_D$ value for the prosapogenin was calculated from our

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rotation in pyridin; ELLIOTT et al. value[5] for quillaic acid was used for the aglycon.

Although there is no direct proof that the glucuronic acid is linked to the carbon 3 hydroxyl, it can be fairly well accepted on phytochemical grounds[13].

[13] Acknowledgments. We thank Ldo. T. WEIL for his help and Prof. D. H. R. BARTON (London) for a gift of the methyl ester of quillaic acid.

Résumé. La prosapogenine obtenue de la quillaja saponine est le β-D-glucopyranuronoside de l'acide quillaïque.

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The Effect of Uncouplers of Oxidative Phosphorylation on Sodium Transport in the Proximal Renal Tubule of the Rat

The question of the source of energy for the postulated active transport and reabsorption of sodium from the proximal mammalian tubule has not been elucidated. In the toad bladder, frog skin[1] and even in the proximal tubule of Necturus[2], on the other hand, oxidative phosphorylation has been shown to play a significant role in the energetics of sodium transport. Experiments with inhibitors of oxidative phosphorylation 2,4-dinitrophenol and oligomycin in dogs[3–5] and rats[6] led to the conclusion that in the dog kidney the energy for sodium transport is not derived from ATP. This conclusion is contra-indicated by the finding of KATZ and EPSTEIN[7] on the direct relationship between Na-ATPase activity and sodium reabsorption in rat kidneys, and that of CHERTOK et al.[8] who observed a slight inhibition of proximal reabsorption after applying DNP into the renal tubule.

In the present work, we used in addition to DNP, the highly effective uncoupler of oxidative phosphorylation from the group of carbonyl cyanide phenyl hydrazones (their *p*-trifluoromethoxy derivative, FCCP) which, in a way similar to DNP, causes hydrolysis of intermediate products and oligomycin which interferes with the final phase of ATP synthesis.

The intrinsic reabsorptive capacity was measured in the proximal tubules of albino rats using the shrinking droplet technique as described by GERTZ[9]. All inhibitors were applied into the tubular lumen.

A concentration of $10^{-4}M$ DNP caused inhibition of reabsorptive capacity of the proximal tubule corresponding to the finding of CHERTOK et al.[8] both in acid and neutral solutions. The same inhibition was attained by administering $10^{-7}M$ FCCP while a concentration of $10^{-4}M$ FCCP induced 60% inhibition. The effect of oligomycin (2 γ/ml) was considerably increased after 4 applications to the same site in the tubule (so-called cummulative dosis). This might be related to its affinity for the protein structures of membranes[10].

These findings support the opinion that ATP might be the direct source of energy for sodium reabsorption in the proximal tubule of the kidney also in mammals. The negative results mentioned[3–6] obtained in experiments with the whole animal, might be related to the fact that inhibitors were applied via the blood. It was observed in experiments with isolated mitochondria that albumin added to the medium decreases the effect of DNP. Oligomycin is bound to the protein structures of membranes, and this may play a role when it passes through the system of membranes from the capillaries to the site of action inside the tubular cell when applied into the blood. A definite answer as to the role of ATP in proximal fluid absorption must wait for further experiments in which uncouplers of oxidative phosphorylation might also act from the interstitial side.

Zusammenfassung. Durch direkte intratubuläre Applikation von Entkopplern und Antagonisten der ATP wird wahrscheinlich gemacht, dass ATP bei der aktiven Rückresorption von Na im proximalen Konvolut der Ratte eine entscheidende Rolle spielt.

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Control	2,4-Dinitrophenol ($10^{-4}M$)		Oligomycin (2 γ/ml)	
	pH 5.8	pH 7.3	Single[a] dosis	Cumulative[a] dosis
$t^1/_2$				
9.1 ± 1.37	11.9 ± 2.05	11.4 ± 2.13	11.3 ± 1.74	14.1 ± 1.92
Control	FCCP			
	$10^{-3}M$	$10^{-4}M$	$10^{-5}M$	$10^{-6}M$
$t^1/_2$				
8.7 ± 1.17	23.2 ± 2.61	17.3 ± 2.04	15.2 ± 2.31	13.1 ± 1.95

$t^1/_2$, Half-time of oil shrinkage; values presented as mean ± residual standard error. [a] See text.

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