Das Auftreten von Koproporphyrin in Kulturen von *Poteriochromonas stipitata* nach Inkubation mit 3-Amino-1,2,4-triazol (Amitrol)

Bei Untersuchungen über den Einfluss von Amitrol auf die Pigmentbildung in der einzelligen Alge *Poteriochromonas stipitata*[1,2] wurde beobachtet, dass sich die Kulturen nach Zusatz von mehr als $2 \cdot 10^{-3} M$ Amitrol, parallel zur Abnahme des Chlorophyllgehaltes, zunehmend rostrot verfärbten.

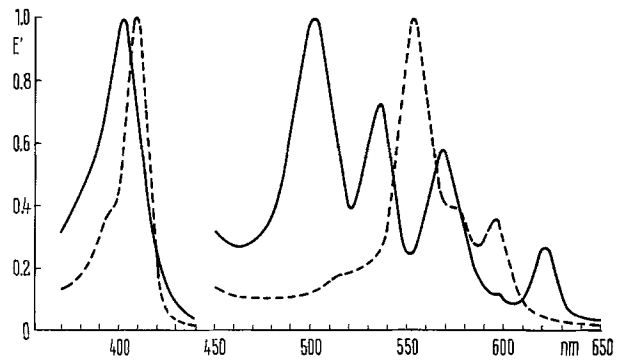

Fig. 1. Absorptionsspektrum des aus amitrolbehandelten Kulturen von *P. stipitata* isolierten Porphyrins. ———, in Chloroform; – – –, in 25%iger HCl. Für die Messung des Absorptionsspektrums im Bereich von 450–650 nm wurde eine Porphyrinlösung benutzt, die 10–20mal so konzentriert war wie die im Bereich von 370–440 nm verwendete.

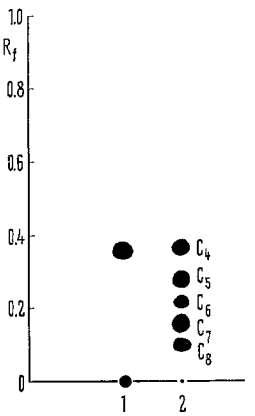

Fig. 2. Dünnschichtchromatographische Identifizierung des Porphyrins. Schicht: Kieselgel G. Laufmittel: Benzol-Äthylacetat-Eisessig (85:13:2). Laufzeit: ca. 45 Min für 12,5 cm. Aufgetragen: 1. Methylester des Porphyrins aus amintrolbehandelten Algenkulturen; 2. Methylester der Porphyrine aus dem Harn eines Patienten mit Porphyria cutanea tarda; C = Anzahl der Carboxylgruppen.

Der rote Farbstoff konnte mit Äthylacetat-Eisessig (3:1) sowohl aus den Algenzellen als auch aus dem Nährmedium extrahiert werden. Sein Absorptionsspektrum entsprach dem eines Porphyrins vom Ätiotyp (Figur 1). In Chloroform lagen die Absorptionsmaxima bei 403, 501, 536, 539 sowie 622 nm und in 25%iger HCl bei 409, 553, 573 sowie 596 nm. Die dünnschichtchromatographische Identifizierung des Porphyrins bereitete zunächst gewisse Schwierigkeiten, da es sich auf übliche Weise[3] nicht methylieren liess. Erst nach vorheriger Hydrolyse mit methanolischer KOH war das Ergebnis der Methylierung zufriedenstellend. Als möglicher Grund hierfür wurde eine Maskierung der Carboxylgruppen angenommen. Auf Kieselgel-G-Schichten mit Benzol-Äthylacetat-Eisessig (85:13:2) als Laufmittel[4] hatte das methylierte Porphyrin den gleichen Rf-Wert wie Koproporphyrintetramethylester (Figur 2).

Der Nachweis von Koproporphyrin in den mit Amitrol inkubierten *P.-stipitata*-Kulturen überraschte insofern, als bisher weder in amitrolbehandelten Pflanzen noch in amitrolbehandelten Algen das Vorkommen von Porphyrinen beschrieben wurde. Dieser Befund deutet an, dass die durch Amitrol bedingte Hemmung der Chlorophyllsynthese in *P. stipitata* ausser in der gestörten Chloroplastenentwicklung[5] möglicherweise auch in einer Beeinflussung der Porphyrinsynthese zu suchen ist.

Summary. In the presence of more than $2 \times 10^{-3} M$ amitrole, a red pigment was synthesized in the unicellular alga *Poteriochromonas stipitata*. By means of TLC it was confirmed that it consists mainly of coproporphyrine.

P. DÖRFLING, W. DUMMLER und D. MÜCKE

Institut für Physiologische Chemie der Universität Rostock, 25 Rostock 1 (DDR), 5. Januar 1970.

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Human Skin-Surface Lipid Fatty Acids – Mosquito Repellents

Human skin-surface lipids have been found to contain components repellent to female *Aedes aegypti* mosquitoes when evaluated in a dualport olfactometer[1]. The hydrocarbon fraction of these lipids was isolated and found to contain weakly repellent unsaturated hydrocarbons[2]. The major repellent compounds, however, were present in the more polar fractions. We have now isolated the free fatty acid fraction from skin-surface lipids that were obtained from ether washings of the elbows of a number of individuals. This fraction was isolated from pooled lipids by short-path vacuum distillation at 100 °/0.025 mm Hg, followed by separation on silica gel GF thin-layer chromatography (TLC) using benzene as the developing solvent. The spot at Rf, 0.7 (42 mg), consisted of mixed fatty acids. Further fractionation of these fatty acids was obtained by the use of gas-liquid chromatography (GLC) as shown in the Figure. Repeated injection into the GLC and condensation of the eluates yielded the 4 fractions re-

ported in Table I. Fraction III consisted primarily of *n*-C_{14} fatty acid as evidenced by comparison of the IR-spectrum and GLC behavior with those of an authentic sample. Fraction II was identified by GLC, NMR- and mass-spectrometry as isotetradecanoic acid. Fraction IV consisted of 2 peaks, which were identified by comparison of GLC behavior with that of authentic samples of *n*-pentadecanoic acid (15 min), and *n*-hexadecanoic acid (20 min). It was recognized that any unsaturated fatty acids present in these fractions at low concentrations would be buried under the larger peaks of the saturated fatty acids that are present in larger amounts.

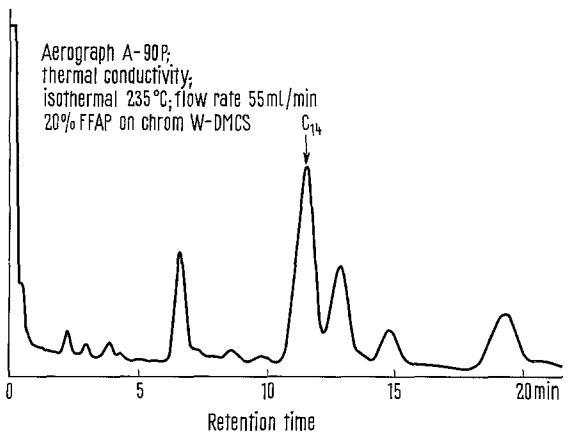
In order to determine whether saturated fatty acids were responsible for the repellency of these fractions, a series of authentic fatty acids were evaluated in the dual-port olfactometer, using the previously described procedure[1]. These results are seen in Table II. It can be seen that the peaks of fraction IV, consisting mainly of *n*-pentadecanoic and *n*-hexadecanoic acids could not account for the repellency noted (Table I) in that fraction. Also, the repellency of isotetradecanoic acid cannot account for the repellency of fraction II.

The mixture of fatty acids (Rf, 0.7) isolated from TLC was converted to methyl esters by treatment with diazomethane, and the methyl esters were chromatographed on TLC (silica gel G–15% $AgNO_3$) and developed with benzene:ether (2:1). This resulted in separation into 3 spots which could be detected by H_2SO_4 spray (Rf, 0.68, 0.43 and 0.00). The amounts recovered from 20 mg of original mixed methyl esters were 7.0 mg, 4.0 mg and 2.0 mg. A portion of the least polar fraction (Rf, 0.68) was injected into the GLC and gave a pattern of peaks similar to that in the Figure. The NMR-spectrum of this fraction showed it to be a mixture of methyl esters of saturated fatty acids. Hydrolysis of this fraction to recover the free acids and evaluation in the dual-port olfactometer for repellency to female *Aedes aegypti* indicated it to be only weakly repellent (IR = 7).

Since not enough of the unsaturated fractions (Rf, 0.43 and 0.00) were available for hydrolysis and evaluation in the olfactometer, a portion of molecularily distilled skin-surface lipids was dissolved in ether and extracted with 1% NaOH to remove acidic components. This extract was then acidified with hydrochloric acid and extracted with ether to isolate the free fatty acid mixture. Evaluation in the dual-port olfactometer showed an average of 5.9 mosquitoes on the sample side out of a total of 20. This repellent fraction was esterified and chromatographed using TLC (silica gel G–15% $AgNO_3$) and eluted with benzene–ether (2:1). Three fractions were detected at Rf, 0.73, 0.54 and 0.00. NMR showed the first fraction to be saturated fatty acid esters and the second and third to be unsaturated fatty acid esters. After basic hydrolysis, these 3 fractions were evaluated as free acids in the dual-port olfactometer. The saturated portion was not significantly repellent while the unsaturated portions showed 2.5 and 5.0 mosquitoes on the sample side out of a total of 20 mosquitoes in the cage.

A series of authentic unsaturated fatty acids then were evaluated as repellents to female *Aedes aegypti* in the dual-port olfactometer; the results are seen in Table III.

The literature on human skin-surface lipid free fatty acids indicates that straight-chain members of both odd and even carbon numbers are present and that the predominant position of the double bond of the monoenes is between the 6th and 7th carbon atoms from the carboxyl group[3]. NICOLAIDES[4] investigated the free fatty acids in human scalp lipids and concluded that the double bonds



VPC separation of TLC fraction (Rf, 0.7) of vacuum-distilled skin-surface lipids.

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Table I. Characteristics of vapor phase chromatographic components of TLC fractions (Rf 0.7) of vacuum-distilled skin-surface lipids[a]

	Fraction I	Fraction II	Fraction III	Fraction IV
VPC Components, Wts (mg)	4.5	2.1	6.5	2.6
VPC Retention time (min)	1–5	8	13, 14	15, 20
Repellency data[b]	6.9	6.1	8.6	4.1
IR, λ_{max}^{NaCl} cm^{-1}; OH	3400			
bonded OH		3600-2500	3700–2500	3700–2400
C=O	1640	1720	1700	1700
other	1110	720	1290, 720	720

[a] Skin-surface lipids were vacuum-distilled first by short-path and then through the Vigreux column. [b] Number of mosquitoes on sample side out of a total of 20 mosquitoes.

Table II. Repellency of saturated fatty acids to female *Aedes aegypti* mosquitoes in a dual-port olfactometer

Fatty acid (100 mg/sample)	No. of carbon atoms	Repellency data[a] *IR*	Average *IR*
Pentanoic	5	55, 30	43
Hexanoic	6	44, 23, 78, 48, 54	59
Heptanoic	7	34, 53, 80, 77	61
Octanoic	8	40, 38, 55, 62	49
Nonanoic	9	80, 39, 76, 69, 59, 57	63
Decanoic	10	30, 55, 49	45
Undecanoic	11	41, 68, 67, 43, 41	52
Dodecanoic	12	9, 19, 46, 67, 30, 39	35
Tridecanoic	13	20, 43, 32	32
Tetradecanoic	14	—3, 3, —5, —11, 20, —18	—2
Isotetradecanoic	14	2, —4, 25, 5	7
Pentadecanoic	15	14, 23, 27, 31	24
Anteisopentadecanoic	15	35, —9, 46, 13	21
Hexadecanoic	16	19, —1, —12, 8	4
Isohexadecanoic	16	—1, —8, 2, 9, —8	—1
Heptadecanoic	17	6, 39, 16, 31	23
Anteisoheptadecanoic	17	—6, 7, 1, 7, 7	3
Octadecanoic	18	1, 3, 14, 17	9

[a] Repeat experiments were conducted on different days and with different groups of mosquitoes. *IR* = (No. of mosquitoes on control side–No. of mosquitoes on sample side/Total No. of mosquitoes) ×100.

Table III. Repellency of unsaturated fatty acids to female *Aedes aegypti* mosquitoes in a dual-port olfactometer

Fatty acid (100 mg/sample)	No. of C=C	No. of carbon atoms	Repellency data[a] *IR*	Average *IR*
2-Nonenoic	1	9	51, 50, 70, 59	57
2-Decenoic	1	10	52, 75, 76, 53	64
10-Undecenoic	1	11	46, 53, 58, 60	54
2-Dodecenoic	1	12	54, 58, 59, 49, 29	49
9-Tetradecenoic(cis)	1	14	4, 17, 33, 28	20
9-Hexadecenoic(cis)	1	16	—1, 16, 29	15
9-Hexadecenoic(trans)	1	16	13, 7, 10, 0	10
9-Octadecenoic	1	18	40, 12, 52, 12, 35	30
13-Docosenoic(cis)	1	22	66, 20, 19, 23	32
15-Tetracosenoic(cis)	1	24	59, 43, 23	42
9,12-Octadecadienoic	2	18	31, 30, 31, 26	30
9,12,15-Octadecatrienoic	3	18	41, 79, 72, 89, 84, 62	71
5,8,11,14-Eicosatetraenoic	4	20	71, 39, 59	56

[a] Repeated experiments were carried out on different days with different groups of mosquitoes. *IR* = (No. of mosquitoes on control side–No. of mosquitoes on sample side/Total No. of mosquitoes) ×100.

of all the fatty acids, including straight as well as branched chained members are located either in the 6,7 position or are removed from this position by an integral number of C_2 units, with the one exception of oleic acid. Dienes in chain lengths from C_{18} to C_{24} (about 3% of the total free fatty acids) as well as trace amounts of trienes and tetraenes[5], were also present. Branched unsaturated fatty acids make up about 4% of the total free acids present. In the case of the monoenes, the lowest carbon chain detected was C_{14} and with the saturated fatty acids none were detected with carbon chain lengths below C_{13}. A survey of the data in Tables II and III would indicate that the repellency of the free fatty acid fraction present in skin-surface lipids must be accounted for on the basis of the unsaturated acids present.

In future studies, efforts will be made to reduce the attraction of animals to mosquitoes by increasing the amounts of unsaturated fatty acids present in their skin lipids[6].

Résumé. On a découvert que les lipides de l'épiderme humain sont répulsifs pour la femelle d'*Aedes aegypti*. Cet effet est dû en partie à la présence d'acides gras non saturés. Les acides gras saturés ne contribuent pas à cette action.

W. A. SKINNER and H. C. TONG;
H. I. MAIBACH and D. SKIDMORE

Life Sciences Research, Stanford Research Institute, Menlo Park (California 94025, USA); and University of California Medical School, Division of Dermatology, Department of Medicine, San Francisco (California, USA), 24 December 1969.

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