Proposed nomenclature of spinochromes

Suffix	m. p.	Formula	Previous name	Origin	
A	185°	$C_{12}H_{10}O_8$	Spinochrome	North Atlantic *P. lividus*	LEDERER and GLASER[1] GOODWIN and SRISUKH[2]
B	>300° *	$C_{12}H_8O_7$ **	Spinochrome P_1	*P. lividus* from Atlantic or Mediterranean	MUSAJO and MINCHILLI[3] GOODWIN and SRISUKH[2]
C	247°	$C_{12}H_8O_8$	iso-echinochrome; perhaps identical with spinon *A* .	*Arbacia pustulosa* and *P. lividus* Mediterranean	GLASER and LEDERER[4] KUHN and WALLENFELS[5]
D	295°		Spinochrome *Aka*	*Pseudocentrotus depressus*	KURODA and OSHIMA[6]
E	>350°		new pigment	*P. lividus* Mediterranean	LEDERER[7]
F	229°		Spinochrome *F*	*Heterocentrotus mammilatus*	KURODA and OSHIMA[6]
G	>350°		new pigment	*P. lividus* Mediterranean	LEDERER[7]
M	193°		Spinochrome *M*	*Anthocidaris crassispina*	KURODA and OSHIMA[6]
P	188°	$C_{12}H_{10}O_7$	Spinochrome *P*	*P. lividus* Mediterranean	MUSAJO and MINCHILLI[8]

* MUSAJO and MINCHILLI found m. p. above 350°, GOODWIN and SRISUKH[2] found m. p. 283°. Our recent preparations melt above 300° with decomposition and partial sublimation. It is very difficult to state a definite melting point.

** This is the formula suggested by MUSAJO and MINCHILLI[3] who obtained four concordant analyses.

[1] E. LEDERER and R. GLASER, C. r. Acad. Sci. Paris *207*, 454 (1938).

[2] T. W. GOODWIN and S. SRISUKH, Biochem. J. *47*, 69 (1950).

[3] L. MUSAJO and M. MINCHILLI, Bol. sci. Fac. Chim. Ind. Bologna *3*, 113 (1942).

[4] R. GLASER and E. LEDERER, C. r. Acad. Sci. Paris *208*, 1939 (1939).

[5] R. KUHN and K. WALLENFELS, Ber. dtsch. chem. Ges. *74*, 1594 (1941).

[6] C. KURODA and H. OSHIMA, Proc. Imp. Acad. Tokyo *16*, 214 (1940).

[7] E. LEDERER, Biochim. biophys. acta (1951) (in press).

[8] L. MUSAJO and M. MINCHILLI, Gazz. Chim. Ital. *70*, 287 (1940).

LEDERER and GLASER[1] first reported in *Arbacia* without specifying the site of occurrence, was assumed from its name to occur in the eggs. LEDERER[2], however, had pointed out in his review that this pigment does in fact occur in the spines of *Arbacia*, and has since confirmed this[3]. Isoechinochrome, which is probably identical with Spinon *A*[4] is thus renamed spinochrome *C*. Although recent work has confirmed the view of GOODWIN and SRISUKH[5] that spinochrome P_1 and *B* are identical, it has shown quite conclusively that spinochromes *A* and *P* are distinct pigments in spite of very similar properties[3]. The suffix *P* is therefore retained for the pigment of MUSAJO and MINCHILLI[6].

Spinochrome *AKa*, isolated from Japanese urchins[7] has been renamed *D;* spinochrome *F* cannot, however, be as KUHN and WALLENFELS[4] suggest, spinochrome *A*, because of the very great differences in m. p.

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Résumé

La présente note contient une proposition de nomenclature des pigments naphtoquinoniques des parties calcaires d'Oursins. Les différents spinochromes sont désignés par des lettres majuscules, *A*, *B*, *C*, etc. Le tableau donne cette nomenclature.

[1] E. LEDERER and R. GLASER, C. r. Acad. Sci. Paris *207*, 454 (1938).

[2] E. LEDERER, Biol. Rev. *15*, 273 (1940).

[3] E. LEDERER, Biochem. biophys. acta (1951) (in press).

[4] R. KUHN and K. WALLENFELS, Ber. dtsch. chem. Ges. *74*, 1594 (1941).

[5] T. W. GOODWIN and S. SRISUKH, Biochem. J. *47*, 69 (1950).

[6] L. MUSAJO and M. MINCHILLI, Gazz. Chim. Ital. *70*, 287 (1940).

[7] C. KURODA and H. OSHIMA, Proc. Imp. Acad. Tokyo *16*, 214 (1940).

Arenicochrome, a new Pigment from *Arenicola marina* L.

In a histological and histochemical investigation LIGNAC[1] some years ago described a pigment occurring as green granules in the epithelial cells of the worm *Arenicola marina* L. found off the Dutch coast at Den Helder.

This pigment, which was called arenicochrome by LIGNAC, showed some analogy to the melanins when judged by its granular form (0·25 to 1·30 μ) and localization within the cell. On treating a tissue-section with hydrogenperoxide the arenicochrome granules could be transformed into brown granules which in turn were made black by silvernitrate and could be decolourized by further treatment with hydrogenperoxide. The two latter reactions are also given by the melanins, so that it seemed that arenicochrome could be considered as a premelanin.

A search of the available literature revealed that the only two authors dealing with pigments in the skin of *Arenicola* species (FAUVEL[2] and ASHWORTH[3]) had probably not observed these green granules in their material.

The pigment could be extracted from the skin of *Arenicola* by means of an alkaline extraction-fluid but it proved to be very unstable in the crude extract. We

[1] G. O. E. LIGNAC, Proc. Kon. Acad. Wetensch. Amsterdam *48*, 406 (1945).

[2] P. FAUVEL, C. r. Soc. Biol. *129*, 1273 (1899).

[3] J. H. ASHWORTH, *Arenicola* (*The lugworm*) (London, 1904).

succeeded in isolating extremely small amounts of crystalline salts of arenicochrome by means of chromatography on aluminiumoxide of an ammonia-alcohol extract. The preparation of a quantity sufficient to permit analysis was successfull only when extensive initial experiments had indicated that the stability of the pigment in the extracts was markedly increased by the addition of cyanide. The blue potassium salt of arenicochrome in particular proved to crystallize beautifully and could be further purified by recrystallization.

We were led to assign the formula $(C_{21}H_{15}S_2O_{14}K_3)_n$, where $n = 1$ or $n = 2$, to this salt on the strength of the analytical results. The procedure adopted finally made it possible to prepare some 50 mg of the potassium salt of arenicochrome, starting from some one thousand worms.

The manner in which the sulphur was bound in the molecule became clear in the course of further investigations, since it appeared that arenicochrome was relatively stable in alkaline solution, in contrast to its behaviour in a weak acid solution. In the latter instance decomposition took place even at room temperature, sulphur being eliminated as sulphuric acid. This phenomenon, which was checked by quantitative determinations, is strongly reminiscent of the behaviour of the sulphuric acid esters of phenols which, moreover, are also known to yield crystallized potassium salts easily. From the fact that arenicochrome has the properties of an indicator (change of colour from blue to orange produced by careful acidification) it appeared that one of the three salt forming groups in the molecule was a weak acid group. By studying the absorption spectrum of the compound as a function of the pH, we were able to assign a pK value of 5·7 to 5·8 to this group.

The conclusion that arenicochrome (assuming a molecular weight of approximately 700 for the molecule) contains two strong acid groups (sulphuric acid esters of enolic or phenolic hydroxyl groups) and one group with a pK value of about 5·7, was confirmed by the successfull preparation of a mixed calcium-potassium salt, which had the formula $(C_{21}H_{15}S_2O_{14}KCa \cdot 3H_2O)_n$ and an orange coloured acid potassium salt the composition of which corresponded more or less to $(C_{21}H_{16}S_2O_{14}K_2)_n$.

It was possible to isolate the compound which resulted by the elimination of the two sulphuric acid groups from the arenicochrome molecule in the form of dark purple crystals. The behaviour and the analysis of this compound led us to believe that we had obtained it in a state of chemical purity. We propose to call this substance arenicochromine. It probably has the composition $C_{42}H_{34}O_{18}$ (or perhaps $C_{40}H_{32}O_{15}$) which has been determined from a number mutually corresponding analyses obtained from a set of independently prepared samples.

The fact that arenicochromine still contains an acid group with pK about 6, is indicated by a change of colour from red to green which takes place when this point is reached on the addition of alkali. Instead of the sulphuric acid groups present in arenicochrome itself, arenicochromine contains one or more weak acid groups with pK values of 9 or higher, the dissociation of which is accompanied by a change of colour from green to purple.

Arenicochromine is soluble in alkaline alcohol-water mixtures as well as in acetone, pyridine, glacial acetic acid and formic acid. It is insoluble in water, methanol, ethanol, amylalcohol, ether, cyclohexanol, chloroform, petroleum ether, carbondisulfide and benzene.

In connection with the properties of arenicochrome and arenicochromine it is important to mention that both pigments in alkaline solvents can be reduced with $Na_2S_2O_4$ to a yellow coloured solution, the colours of which return to blue and green respectively on shaking with air. A typical blue halochromic effect was produced by dissolving the substances in concentrated sulph. acid.

All these results point towards the fact that arenicochrome and arenicochromine are not identical with any known and well defined pigments described in the literature at our disposal. The question arises whether it would be at all possible to class them among any of the existing nitrogen free classes of pigments found in nature. Some superficial similarity to the nitrogen containing ommochromes[1] (being better called insectorubin according to recent investigations[2]) is evident. It is interesting to note that these pigments also seem related to the melanins. The pigments calliactine[3] and hallachrome[4] also show a superficial relationship to arenicochromine.

Amongst the nitrogen-free pigments the echinochromes and spinochromes[5], as far as we are aware, show relatively the most pronounced resemblance, although here the insolubility of arenicochromine in ether and benzene as well as its blue halochromic effect with concentrated sulphuric acid are counter indications to the existence of a more intimate relationship.

We have reason to suggest that some correlation exists between the facts that arenicochrome contains about 10% sulphur, and that this pigment has been obtained in relatively large quantities from animals living in mud which contains a high percentage of FeS. It would be interesting to know whether the occurence of arenicochrome in the skin of *Arenicola marina* L. is restricted to those worms living in mud similar to that found at Den Helder.

The green colour and granular nature of the arenicochrome in the tissue-section can be explained by the assumption that arenicochrome is bound in the cell to some cytoplasmatic component of a granular nature, accompanied by the colourshift from blue to green. The nitrogen-free arenicochrome cannot be a precursor of the known mammalian melanins as these contain about 7 to 9% nitrogen. The possibility cannot at present be excluded that more than one precursor of the melanins exists. In addition we must stress our ignorance concerning the chemical composition of the melanin of *Arenicola marina* L. as well as of other invertebrates.

Full details of this investigation have been published in Dutch[6] and will soon be published in English[7].

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Biochemical Department of the Pathological Laboratory, State-University of Leyden, June 15, 1951.

Zusammenfassung

Das von LIGNAC histologisch in *Arenicola marina* L. gefundene Pigment (= Arenicochrom) konnte als Kaliumsalz in kristalliner Form isoliert werden. Die Analysen ergaben die Formel $(C_{21}H_{15}S_2O_{14}K_3)$ (n = 1 oder 2).

Der Schwefel läßt sich sehr leicht durch saure Hydrolyse als Schwefelsäure abspalten. Der schön kristallisierende schwefelfreie Farbstoff (= Arenicochromin) hat

[1] E. BECKER Z. Indukt. Abst. Vererb. *80*, 157 (1942).
[2] T. W. GOODWIN and S. SRISUKH, Biochem. J. *47*, 549 (1950).
[3] E. LEDERER, G. TEISSIER, and C. HÜTTRER, Bull. Soc. Chim. *7*, 608 (1940).
[4] J. D. BU'LOCK, J. HARLEY-MASON, and H. S. MASON, Biochem. J. *47*, XXXII (1950).
[5] T. W. GOODWIN and S. SRISUKH, Biochem. J. *47*, 69 (1950).
[6] P. v. DUIJN, Thesis (Leiden 1951).
[7] P. v. DUIJN, Rec. Trav. Chim., Pays-Bas (to be published).

die Zusammensetzung C_{42} H_{34} O_{16} oder C_{40} $H_{32}O_{15}$. Die bisher bekannten Eigenschaften lassen eine Einordnung in eine der bekannten Farbstoffklassen nicht zu. Der mögliche Zusammenhang des Arenicochroms mit dem Schwefelgehalt des Milieus, in welchem Arenicola lebt, wird betont. Die Beziehung zur Melaninfrage wird kurz diskutiert.

The Influence of Amino Acids on Growth and Lateral Root Formation in Cotyledon-less Pea Seedlings

It is a well known fact that the early development of most seedlings is controlled to a large extent by the material present in the cotyledons or the endosperm. Not only does this material provide a most important source of energy during a period when the young plant is capable of only a very limited photosynthesis, but it also influences, in a hitherto unexplained way, the differentiation of the seedling. One of the most striking effects of a removal of the cotyledons is the complete change which often occurs in the order and place of development of the lateral roots (RIPPEL[1]). This phenomenon, which applies particularly to some leguminous plants, is analysed in the present study with pea seedlings as the test object.

In normal pea seedlings, cultivated as described below, most if not all of the lateral roots develop in the upper half of the primary root, in cotyledon-less seedlings in the lower half. In the former case the "average insertion point" (A.I.P) of the laterals is situated at circa 25% of the distance between the root-neck and the root-tip, while in the latter case this point is at 50–60% of the same distance. These values refer to seedlings with a primary root about 10 cm long, grown in sterile tube cultures, each containing 10 ml nutrient agar (with 2% sucrose) and continuously illuminated at + 25°C.

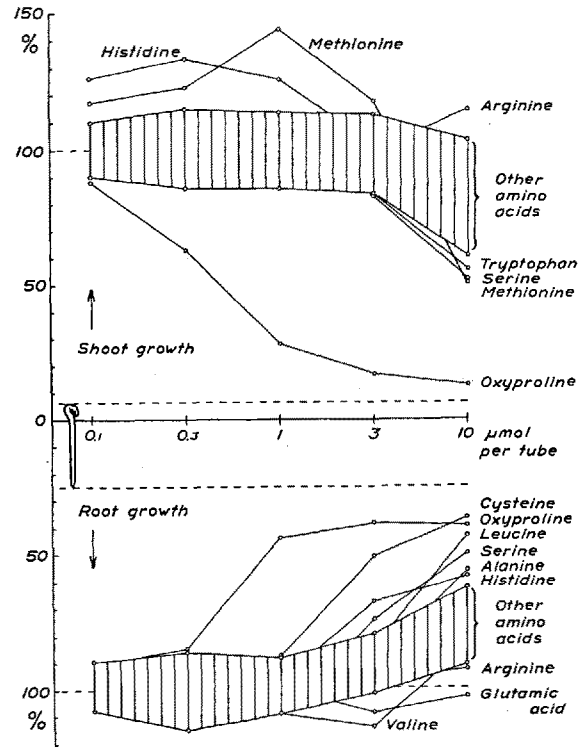
A great part of the substance or substances responsible for the effect are easily washed out from the cotyledons by soaking the peas in sterile water during at least two days before germination. Seedlings developed from peas soaked in this way resemble the decotyledonized seedlings. On the other hand, by adding pea exudate or yeast extract to the culture medium of decotyledonized seedlings, these adopted an appearance more or less similar to that of normal seedlings. Thus, it seemed possible that the substance or substances producing the shift in A.I.P. of the lateral roots are capable of acting in much the same way when absorbed from the medium as when passing from the cotyledons down through the root.

Since amino acids as well as certain nucleic acid derivatives are known to occur in exudate from peas and roots of pea seedlings[2], it seemed justified to test the effect of such substances. In all these experiments each compound and concentration used comprised 6 (in some cases 5) cultures, and the incubation time was 15 days.

As can be seen in the figure, all amino acids, except glutamic acid and probably arginine, were more or less inhibitory to root growth in a concentration of 10 μmoles per 10 ml of nutrient medium, some of them in even lower concentrations. By far the most inhibiting was oxyproline, which reached its maximal effect slightly above 1 μmole/tube. Valine alone seemed to produce a certain promotion of root growth in a concentration of 3 μmole/tube.

[1] K. RIPPEL, Ber. Dtsch. bot. Ges. *55*, 288 (1937).
[2] N. FRIES and B. FORSMAN, Physiologia Plantarum *4*, 410 (1951).

The shoot was less sensitive to the amino acids added, except in the case of oxyproline, the inhibitory effect of which was obvious even in amounts below 0·3 μmole/tube. Three amino acids, viz. histidine, methionine, and arginine, appeared to act favourably on shoot growth, although at different concentrations.



The effect of various concentrations of amino acids on increase in length of shoot and root of cotyledon-less pea seedlings during 15 days, expressed as per cent of control. Amino acids tested: *dl*-alanine, *l*-arginine, *l*-asparagine, *dl*-aspartic acid, *l*-citrulline, *dl*-cysteine, *l*-glutamic acid, glycine, *l*-histidine, *l*-isoleucine, *l*-leucine, *l*-lysine, *dl*-methionine, *l*-ornithine, *l*-oxyproline, *l*-phenylalanine, *l*-proline, *dl*-serine, *dl*-threonine, *l*-tryptophan, *l*-tyrosine, and *l*-valine.

Of the 22 amino acids tested only arginine influenced the formation of lateral roots in a way similar to that of the above mentioned extracts, 10 μmoles per culture causing a change in the A.I.P. value from 57% to 25% (see the table). Analogous, but weaker and more irregular effects were produced by valine, histidine, citrulline, and ornithine. No effect was obtained with yeast nucleic acid, adenine, guanine, hypoxanthine, uracil, urea, creatine, inositol, choline, ascorbic acid, thiamin, pyridoxin,

The effect of arginine and yeast extract on the "average insertion point" (A.I.P.) of the lateral roots in decotyledonized pea seedlings

Experim. No.	Addition per culture (10 ml)	Relative length		A.I.P., % (± standard error)
		Shoot	Root	
948	None	100	100	56·7 ± 2·5
	Arginine, 1 μmole	106	101	60·6 ± 1·9
	Arginine, 3 μmole	100	98	45·3 ± 3·3
	Arginine, 10 μmole	115	93	25·4 ± 4·1
973	None	100	100	57·0 ± 2·7
	Yeast extract, 1 mg	86	99	56·7 ± 2·3
	Yeast extract, 1 mg	95	100	41·6 ± 3·4
	Yeast extract, 10 mg	104	99	23·9 ± 3·0