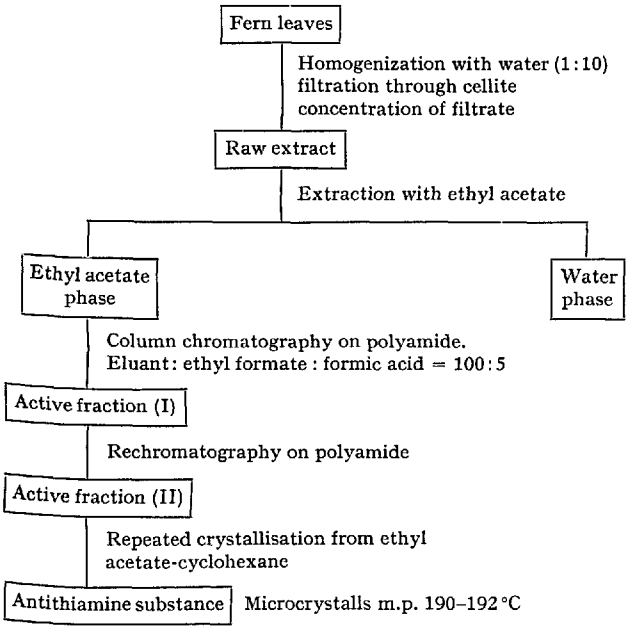

Fig. 1. Scheme of the isolation of a thermostable antithiamine factor of fern.

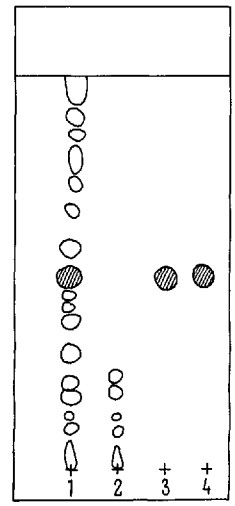

Fig. 2. Thin-layer chromatogram of different stages of the purification. (1) Ethyl acetate phase, (2) water phase, (3) isolated antithiamine substance, (4) 3,4-dihydroxycinnamic acid. Adsorbent: polyamide; developing solvent: ethyl formate: formic acid = 100:5; spray reagent: 1% diphenylboric acid β-amino ethyl ester in methanol.

acetone and ethyl acetate. The microanalyses gave the following results: C, 60.83%; H, 4.57%; O, 33.8%. The molecular weight determined by mass spectrography is 180.

The isolated antithiamine compound of fern was identified as 3,4-dihydroxycinnamic acid by the great agreement of the UV, IR and NMR-spectra, the identical behaviour in thinlayer chromatography (Figure 2), as well as of the other characteristic properties (MP, MW, results of the microanalyses).

The caffeic acid in fern seems to be only partly in free form; another part arises during the extraction (at pH 6.0) from a precursor not yet isolated.

In this connection it is interesting to mention that according to recent experiments (SOMOGYI and BÖNICKE[8]) different *o*-diphenols possess a marked antithiamine activity.

Further investigations to characterise the precursor and to elucidate the mechanism of the antithiamine effect of 3,4-dihydroxycinnamic acid are in progress[9].

Zusammenfassung. Die Isolierung eines Stoffes mit Antithiaminwirkung aus Farnkraut und seine Identifizierung mit 3,4-Dihydroxyzimtsäure wird beschrieben.

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[8] J. C. SOMOGYI and R. BÖNICKE, unpublished experiments 1967.

[9] We thank Dr. R. BÖNICKE, Borstel, for carrying out the microbiological determinations, and Prof. Dr. A. DREIDING, Zurich, for the stimulating discussions. – The microanalyses and IR-spectra have been made by the Institute of Organic Chemistry of the University Zürich and the NMR-spectra by the Institute of Organic Chemistry of the Swiss Federal Institute of Technology (ETH) Zürich and of the University of Zürich.

Utilization of D-Tyrosine by Vertebrate Skin Tyrosinase

During the course of an evolutionary study of vertebrate melanogenesis based upon anatomic and subcellular tyrosinase distribution and activity[1], skin enzyme preparations from several vertebrates were found to utilize D-tyrosine. Skin enzyme preparations were incubated as previously described[2]. When uniformly labeled L-tyrosine-^{14}C or DL-tyrosine-2-^{14}C were used as substrate, the net tyrosinase activity obtained from the latter substrate was higher than that obtained from the former substrate, although the concentration and specific activity of L-tyrosine-^{14}C as well as the enzyme preparations were the same in both series of experiments. As D-tyrosine-^{14}C was not available, the D-tyrosine utilization was determined indirectly by use of L-tyrosine-1-^{14}C, uniformly labeled

[1] Y. M. CHEN and W. CHAVIN, in *Advances in Biology of the Skin* (Ed. W. MONTAGNA; Pergamon Press, Oxford 1967), vol. 8, p. 253.

[2] Y. M. CHEN and W. CHAVIN, Analyt. Biochem. *13*, 234 (1965).

Utilization of D-tyrosine by vertebrate skin tyrosinase

Species	Enzyme preparation[a]	L-tyrosine conversion in T.U.[b]/mg fresh skin ($\bar{x} \pm \sigma_{\bar{x}}$)	D-tyrosine utilized in % of L-tyrosine converted ($\bar{x} \pm \sigma_{\bar{x}}$)
Platyrhinoidis triseriatus (Thorn back ray)	D (1)	104	6.1
	V (1)	53	10.0
Acipenser fulvescens (Lake sturgeon)	W (3)	151 ± 14	4.7 ± 0.3
Amia calva (Bowfin)	D (3)	452 ± 14	11.2 ± 4.4
	V (3)	403 ± 7	–
Lepisosteus osseus (Garpike)	W (3)	28 ± 5	5.7 ± 0.8
Anguilla rostrata (Eel)	D (3)	651 ± 9	6.5 ± 1.7
	V (3)	158 ± 12	10.5 ± 0.4
Necturus maculosus (Mudpuppy)	D (3)	240 ± 21	14.5 ± 2.3
	V (3)	126 ± 6	16.1 ± 1.7
Triturus viridescens (Newt)	D[c]	16,238	5.8
	V[c]	9,085	6.1
Ambystoma tigrinum (Tiger salamander)	D (3)	$2{,}115 \pm 76$	12.7 ± 0.8
	V (3)	$16{,}646 \pm 231$	5.8 ± 0.2
Trionyx ferox (Turtle)	D (3)	153 ± 5	5.4 ± 0.3
	V (3)	46 ± 1	0

[a] D, dorsal skin; V, ventral skin; W, whole skin; No. in parenthesis represents number of animals used. [b] T.U.: 1 T.U. is defined as the amount of tyrosinase activity required to convert 1 picomole of L-tyrosine to melanin under the conditions of the described assay during a 16 h incubation period at 30 °C. [c] Skin of 3 animals combined, due to small size.

L-tyrosine-^{14}C and DL-tyrosine-2-^{14}C. Provided that the quantity and specific activity of the L-form are the same in each of these substrates, the D-tyrosine utilization may be calculated in terms of L-tyrosine converted as follows:

$$\% = \frac{Y' - Y}{A\,Y}\,(100) \quad \text{or} \quad \frac{8\,Y' - 9\,Z + X}{9\,A\,Z - A\,X}$$

where: X is net tyrosinase activity in cpm with L-tyrosine-1-^{14}C, Y is calculated net tyrosinase activity in cpm, representing theoretical L-tyrosine-2-^{14}C value, Y' is net tyrosinase activity in cpm with DL-tyrosine-2-^{14}C, Z is net tyrosinase activity in cpm with uniformly labeled L-tyrosine-^{14}C and A is ratio of specific activities of D-tyrosine in DL-tyrosine-2-^{14}C to that of L-tyrosine in the mixture.

The results of D-tyrosine utilization together with the tyrosinase activity (L-tyrosine conversion) are presented in the Table. In these species the D-tyrosine utilized varies from approximately 5–16% of the L-tyrosine converted (species showing less than 4% were excluded due to the possibility of experimental error). The organisms fall into 4 vertebrate classes: Chondrichthys (thorn back ray), Osteichthys (lake sturgeon, bowfin, eel), Amphibia (newt, salamander, mudpuppy) and Reptilia (turtle). Other species in these classes have been tested and found not to utilize D-tyrosine. In addition, both the phylogenetically more primitive (Agnatha) and the more advanced (Aves, Mammalia) species tested, including man, do not utilize D-tyrosine. It is possible that the species in the Table may oxidatively convert the D-isomer to the α-keto acid followed by L-specific reamination. (Inversion of the D-isomers by racemization has been reported only in bacteria.) The above conversion requires the D-amino acid oxidase. Thus, if D-tyrosine is not directly incorporated into melanin, the findings would indicate the presence of the above oxidase in certain vertebrate integuments. However, as purified mammalian melanoma tyrosinase[3,4] and microsomal *Rana pipiens*[5] tyrosinase preparations utilize D-DOPA and/or D-tyrosine it is probable that the integumental tyrosinase of the species reported herein also utilizes D-tyrosine directly. As the present study has revealed that at least 30% of the vertebrate species tested (9/33) use D-tyrosine directly or indirectly, such utilization may be of significance in melanogenesis. As only very few normal homeothermic vertebrates have been evaluated in regard to the optical specificity of integumental tyrosinase it is expected that future studies will reveal the incorporation of D-tyrosine into melanin in the more highly evolved forms[6].

Résumé. Certaines espèces de vertébrés inférieurs convertissent la D-tyrosine radioactive en mélanine par la tyrosinase de la peau. On pense que l'utilisation normale de la D-tyrosine est importante pour la mélanogenèse intégumentale.

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[6] This investigation was supported by U.S.P.H.S. Grant No. CA-07273-04 GM from the National Cancer Institute and White Laboratories, Inc.