Structures of Adenochromines A and B, the Iron(III) binding Amino-acids of a Unique Group of Peptides, Adenochromes from *Octopus vulgaris*

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Summary Adenochrome, the iron(III)-containing pigment from the branchial heart of *Octopus vulgaris*, has been shown to consist of a group of closely related peptides derived from glycine and two novel iron-binding amino-acids, adenochromines A (**1a**) and B (**1b**), which may arise biogenetically from L-dopa and L-histidine-5-thiol (**2**).

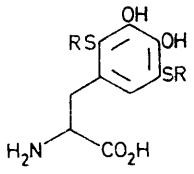
ADENOCHROME,[1] a unique non-porphyrin iron-containing pigment[2] found in the branchial heart of *Octopus vulgaris* has been isolated in the form of deferri-adenochrome (DFA) by acid extraction under conditions capable of reducing Fe^{III} to Fe^{II} (0·5M HCl–2% thioglycolic acid), followed by successive chromatography on Sephadex G-10, Sephadex LH-20, and on Dowex 50W-X8 (yield: 10 mg per g of wet tissue). On acid hydrolysis (3M HCl, 110 °C, 20 h) DFA yielded glycine and two novel iron-binding amino-acids, adenochromines A and B,† in a ratio of *ca.* 2:1, for which we propose structures (**1a**) and (**1b**), respectively.

The adenochromines gave intense green colours with $FeCl_3$, suggesting the presence of an *ortho*-dihydric phenol, in accord with the bathochromic shifts of their u.v. maxima in alkali (Table). Common features of the n.m.r. spectra (2M DCl in D_2O) of the adenochromines were signals from three methylene and three methine groups, one aromatic proton at δ *ca.* 7·0, and two low-field protons around δ 8·8, typical for H-2 of an imidazole ring. Moreover, the n.m.r. spectra exhibited N-Me singlets around δ 4·0 (each $<$ 1H), indicating that adenochromines were partially methylated. These data, coupled with the u.v. spectra (Table), suggested that adenochromines A and B were isomers; adenochromine A hydrochloride analysed for $C_{21}H_{25}O_8N_7S_2 \cdot 5HCl$.‡

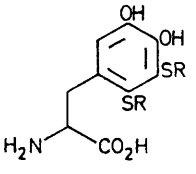
† Hydrolysis of DFA gives also smaller amounts of the third possible isomer (**1c**) (u.v. and n.m.r. evidence) which, however, has not yet been isolated.

‡ The analyses were in agreement with the values calculated by taking into account the 3:1 ratio of NH and NMe homologues. However, for clarity the molecular formulae refer only to the NH homologues.

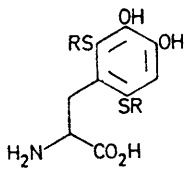
Reductive hydrolysis of both (**1a**) and (**1b**) with 57% hydroiodic acid in the presence of red phosphorus (110 °C, 48 h) gave L-β-3,4-dihydroxyphenylalanine (L-dopa) and a new thiol-containing amino-acid (**2**) in a molar ratio of 1 : 2. The thiol (**2**) was readily oxidised in air to a crystalline disulphide (**3**), $C_{12}H_{16}O_4N_6S_2 \cdot 4HCl \cdot 2H_2O$,‡ which could be reconverted into (**2**) by catalytic hydrogenolysis (Pd–C, 0·1M HCl). Raney nickel desulphuration of (**3**) gave L-histidine and 1-methylhistidine in *ca.* 3 : 1 ratio, reflecting



(1a)

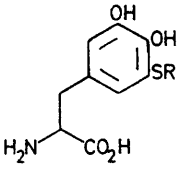


(1b)

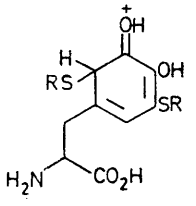


(1c)

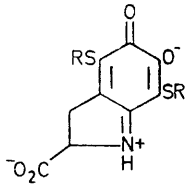
(2) = RSH
(3) = RS-SR



(4)

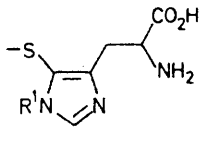


(5)



(6)

RS =



(R¹ = H or Me)

the degree of methylation. When compared with the n.m.r. spectrum of histidine-2-thiol (C-5 proton at δ 7·02), that of (**2**) [δ (2M DCl in D_2O) 3·57 (2H, d, *J* 7 Hz), 3·97 (*ca.* 0·8H, s, N^1-Me), 4·57 (1H, t, *J* 7 Hz), and 8·87 (1H, s)] suggested that it was histidine-5-thiol. A combination of one dopa unit with two of the thiol units (**2**) then led to the general structure (**1**) for adenochromines A and B.

Heating (**1a**) or (**1b**) in 48% hydrobromic acid containing 4% thioglycolic acid gave, presumably *via* protonated species, *e.g.* (**5**), the thiol (**2**), dopa (42%), and secoadenochromine A (37%), $C_{15}H_{18}O_6N_4S \cdot 3HCl$,‡ which was characterized as (**4**) on the basis of its n.m.r. spectrum [δ (2M DCl in D_2O) 3·17 (2H, d, *J* 6·5 Hz), 3·60 (2H, d, *J* 7·5 Hz), 3·96 (*ca.* 0·8H, s, N-Me), 4·37 (1H, t, *J* 6·5 Hz), 4·49 (1H, t, *J* 7·5 Hz), 6·70 and 6·84 (2H, ABq, *J* 1·8 Hz), and 8·82 (1H, s)]. The assignments of the aromatic substitution pattern as in (**4**) was substantiated by the n.m.r. spectra of two related compounds, 5-*S*-cysteinyldopa[3,4] (ABq, δ 6·93 and 7·01, *J* 2·0 Hz) and 6-*S*-cysteinyldopa[4] (singlets at δ 6·94 and 7·20).

TABLE. Absorption spectra of deferri-adenochrome and its hydrolysis products

	λ_{max}/nm		
	pH 1	pH 10	pH 10, after 2—4 h
Deferri-adenochrome	306	320	little change
Adenochromine A (**1a**)	302	318	600
Adenochromine B (**1b**)	306	320	undefined absorption
Secoadenochromine A (**4**)	292	303	570

The formation of (**4**) ruled out structure (**1c**) for adenochromines A and B which are therefore represented by (**1a**) and (**1b**) or *vice versa*. Information on the substitution pattern was provided by a marked difference in the oxidation behaviour of the two isomers. On air oxidation at pH 10 (Table) only adenochromine A is converted into an aminochrome-like pigment (**6**),[5] the formation of which suggests that position 6 is unsubstituted. Furthermore, adenochromine A is more stable than B with respect to the acid cleavage to (**4**), suggesting a less crowded substitution pattern. On this basis we favour structures (**1a**) and (**1b**) for adenochromines A and B, respectively.

Adenochromines may be formed *in vivo* by addition of histidine-5-thiol (**2**) to dopaquinone arising from tyrosine by tyrosinase oxidation. A closely related metabolite, 2,5-*SS*-dicysteinyldopa, has been isolated from the eyes of fish.[6]

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[1] For the history of chemical investigation on adenochrome, see G. Nardi and H. Steinberg, *Comp. Biochem. Physiol.*, 1974, **48B**, 453, and references cited therein.
[2] For a review on non-porphyrin iron-binding compounds, see J. B. Neilands, 'Inorganic Biochemistry,' ed. G. Eichhorn, Elsevier, Amsterdam, 1972, p. 167.
[3] G. Prota, G. Scherillo, and R. A. Nicolaus, *Gazzetta*, 1968, **98**, 495.
[4] S. Ito and G. Prota, *Experientia*, in the press.
[5] For the chemistry of aminochromes, see R. A. Heacock, *Adv. Heterocyclic Chem.*, 1965, **5**, 205.
[6] S. Ito and J. A. C. Nicol, *Tetrahedron Letters*, 1975, 3287.