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2,5-S,S-DICYSTEINYLDOPA: A NEW AMINO ACID IN THE EYE OF THE GAR AND ITS ENZYMIC SYNTHESIS

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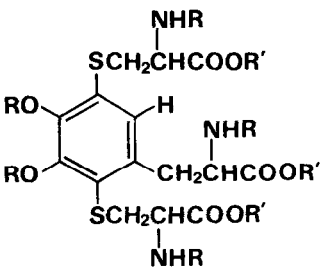
In the course of a study of reflecting materials in fish eyes[1], we have isolated from the alligator gar Lepisosteus spatula a new amino acid whose structure has been established as 2,5-S,S-dicysteinyldopa (Ia).

An acid extract (1M HCl) of 50 eyes was passed on to a Dowex-50W x4 (H^+ form) column and, after washing with 1M HCl, the amino acid was eluted with 6M HCl, and then chromatographed on Sephadex LH-20, using MeOH:1M HCl 19:1 as eluent. The amino acid was further purified by paper chromatography in n-PrOH:1M HCl 3:2 and converted to the formate by passing through a Dowex-2 x8 (formate form) column. Chromatography on Sephadex G-25 in 0.5M HCOOH yielded the amino acid (26 mg) as an amorphous colorless powder. It gave a violet color with ninhydrin and a pale green one with $FeCl_3$ and was pure as judged by t.l.c. on cellulose. The u.v. maxima are given in the Table.

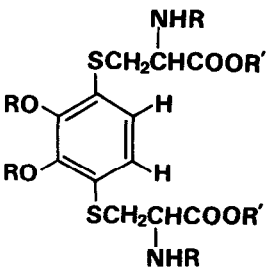
The elemental analysis showed the molecular formula to be $C_{15}H_{21}N_3O_8S_2 \cdot HCOOH$ (Found C, 39.67; H, 5.27; N, 8.76; S, 13.33%; calc.: C, 39.90; H, 4.81; N, 8.73; S, 13.32%). The n.m.r. spectrum (100 MHz, 1M DCl) revealed, besides the formate proton, only ten protons; six methylene protons (δ 3.2-3.8, m), three methine protons (δ 4.3-4.5, m), and one isolated aromatic proton (δ 7.20, s). These data suggested that the amino acid Ia might be derived from one molecule of dopa and two molecules of cysteine. Indeed, reduction with 57% HI-red P[2] (110°, 24 hr) yielded three amino acids, cysteine, dopa (ca. 50%) and 5-S-cysteinyldopa[3] (ca. 20%).

In order to establish the structure and secure much more material, an attempt was made to synthesize the amino acid by enzymic oxidation. Under a carefully controlled

condition (22°, 10 hr), the reaction of L-dopa with L-cysteine (3 eq.) at pH 6.8 in the presence of mushroom tyrosinase and atmospheric O_2 yielded, along with 5-S-cysteinyldopa[3] (68%) and 2-S-cysteinyldopa[4] (5.8%), an amino acid (8.5%), $C_{15}H_{21}N_3O_8S_2 \cdot HCOOH$[5], identical with the gar amino acid in spectral properties (u.v.(Table), i.r., and n.m.r.) and t.l.c. Esterification of the synthetic Ia with MeOH-HCl followed by acetylation with Ac_2O-pyr gave the N, O-pentacetyl trimethyl ester Ib (22%). The n.m.r. spectrum ($CDCl_3$) was consistent with the structure Ib; it revealed the presence of three acetamide groups (δ 1.82, 1.92, 2.04), two phenyl acetate groups (δ 2.37, 2.38), three carbomethoxy groups (δ 3.64, 3.74, 3.78), and one aromatic proton (δ 7.34, s). These results indicated that the amino acid might be 2,5-S,S- or 5,6-S,S-dicysteinyldopa.



Ia R=R'=H
Ib R=Ac, R'=Me



IIa: R=R'=H
IIb: R=Ac, R'=Me

The formation of 2,5-S,S-dicysteinyldopa can be explained by 1,6-addition[3] of the second cysteine molecule to the conjugated o-quinone formed in situ from 5-S-cysteinyldopa, whereas that of 5,6-S,S-dicysteinyldopa can be explained by 1,4-addition of cysteine. That the former is a more likely case was proved by the enzymic synthesis of 3,6-S,S-dicysteinylcatechol, a product of two 1,6-additions. Reaction of catechol with L-cysteine (3 eq.) in the presence of tyrosinase and O_2 gave 3-S-cysteinylcatechol (41%), $C_9H_{11}NO_4S \cdot HCl$[5], m.p. 172-175° (dec.), and 3,6-S,S-dicysteinylcatechol (IIa) (17%), $C_{12}H_{16}O_6S_2 \cdot 2H_2O$[5], m.p. 262° (dec.). The structures of these catechol derivatives were confirmed by their spectral data, especially by n.m.r. spectra.

The presence of three adjacent protons in 3-S-cysteinylcatechol was shown by the n.m.r. spectrum (1M DCl):δ 6.86, dd, J = 8.5, 7.5Hz; 7.01, dd, J = 8.5, 2 Hz, 7.09, dd, J = 7.5, 2 Hz. The n.m.r. spectrum (1M DCl) of 3,6-S,S-dicysteinylcatechol (IIa) indicated that it has a symmetrical structure: two equivalent methylene groups (δ 3.54, d, J = 5.6 Hz) two equivalent methine groups (δ 4.32, t, J = 5.6 Hz) and two equivalent aromatic protons

(δ 7.10, s). Esterification of IIa followed by acetylation yielded the O, N-tetracetyl dimethyl ester IIb (81%); M^+, m/e 544. The symmetrical structure IIb was supported by the n.m.r. spectrum ($CDCl_3$):δ 1.76, 6H, s (NCOMe); 2.40, 6H, s (OCOMe); 3.37, 3.55, both 2H, both dd, J = 14, 4 Hz (CH_2); 3.76, 6H, s (COOMe); 4.88, 2H, dt, J = 7.5, 4 Hz (CH); 6.49, 2H, d, J = 7.5 Hz (NH); and 7.38, 2H, s (aromatic protons).

Table. The u.v. spectral data of the compounds Ia, Ib, IIa, IIb

compound	solvent	λ_{max}/nm (ε)
2,5-S,S-dicysteinyldopa (Ia)	0.1M HCl	217 (23400), 273 (8200), 303 (3100)
(natural)	pH 6.8 buffer	223 (22800), 267 sh. (7200), 316 (3300)
2,5-S,S-dicysteinyldopa (Ia)	0.1M HCl	217 (22700), 273 (8600), 302 (3100)
(synthetic)	pH 6.8 buffer	223 (22100), 267 sh. (6900), 316 (3200)
3,6-S,S-dicysteinylcatechol (IIa)	0.1M HCl	212 (26600), 270 (10800), 298 sh. (3200)
dopa derivative Ib	MeOH	279 (10700)
catechol derivative IIb	MeOH	277 (13200)

Thus, the structure Ia, 3-(2,5-S,S-dicysteinyl-3,4-dihydroxyphenyl)-alanine, can be assigned to the new amino acid from the eye of the gar. The compound may be formed *in vivo* by two consecutive 1,6-additions of two cysteine molecules first to dopaquinone and then to 5-S-cysteinyldopaquinone produced by the action of tyrosinase. 5-S-Cysteinyldopa and 2-S-cysteinyldopa have been shown to be intermediates in the biosynthesis of phaeomelanine[6].

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References and Note

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