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OXIDATION OF PEPTIDYL 3,4-DIHYDROXYPHENYLALANINE ANALOGUES: IMPLICATIONS FOR THE BIOSYNTHESIS OF TUNICHROMES AND RELATED OLIGOPEPTIDES

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ABSTRACT.—The *o*-diphenolic amino acid L-3,4-dihydroxyphenylalanine (dopa), the enamine α,β-dehydro-3,4-dihydroxyphenylalanine (Δ-dopa), and/or hydroxylated derivatives thereof, are integrated into the primary sequence of many scleroproteins and polyphenolic oligopeptides such as the celenamides, tunichromes, and halocyamines. After oxidation of *N*-acetyldopa ethyl ester, a low mol wt analogue of peptidyl dopa, the resultant *o*-quinone tautomerized to (*Z*)-α,β-dehydro-3,4-dihydroxyphenylalanine ethyl ester. We have characterized this Δ-dopa derivative and an acetate 1,4-addition product formed during the synthesis. Tautomerization of peptidyl dopa quinone to Δ-dopa may be involved in the biosynthesis of Δ-dopa-containing oligopeptides.

Clionamide (1), celenamides (2,3), tunichromes (4–7), and halocyamines (8) are structurally related polyphenolic oligopeptides isolated from marine invertebrates as diverse as sponges and ascidians. The tunichromes, isolated from the blood cells of *Ascidia nigra*, may participate in metal binding and/or sclerotization (6), and halocyamines possess antimicrobial activity (8). All integrate the enamines α,β-dehydro-3,4-dihydroxyphenylalanine (Δ-dopa) or α,β-dehydro-3,4,5-trihydroxyphenylalanine (Δ-topa), or their saturated analogues, dopa and topa, in their primary sequence; and each has a C-terminal decarboxy-$\Delta^{2,3}$-unsaturated aromatic residue derived from tyrosine, dopa, topa or 6-bromotryptophan. The dopa moiety of these peptides is also found in, and is crucial to the function of, both various high mol wt scleroproteins isolated from many invertebrates (9–11) and a new class of quino-enzyme which utilizes a peptidyl dopa derivative as an integral redox cofactor (12). Although total syntheses of certain tunichromes have been achieved (7), biosynthetic pathways remain obscure, presumably as a result of the instability of these readily oxidized oligopeptides. Our observation that *N*-acetylDOPA ethyl ester [**1**], an analogue of peptidyl dopa, may oxidize to the *o*-quinone **1a** and then tautomerize (Scheme 1) to the enamide 2-acetamido-3-(3′,4′-dihydroxyphenyl)-2-propenoic acid ethyl ester [**2**], may be pertinent to the biosynthesis of polyphenolic oligopeptides. We report here the structural elucidation of **2**, formed by biomimetic oxidation of **1**, and of a Michael type 1,4-addition byproduct **3**.

Enzymatic 2 electron oxidation (13) of *N*-acetyltyrosine ethyl ester gave compounds **1**, **2**, and **3**. Product **1** was characterized by comparison with L-dopa standards using uv difference spectroscopy in HCl and alkaline borate buffers (14) and, following acid hydrolysis, by both hplc and amino acid analysis. Compound **3** was the 1,4-Michael adduct of acetate with *o*-quinone **1a**. Uv spectroscopy (14) (λ 282 nm, HCl) suggested that **3** was an *o*-diphenol, but the lack of typical chemical reactivity (15–18) indicated that it was disubstituted. The molecular formula of **3**, $C_{15}H_{19}NO_7$, obtained from high resolution fabms (Δ

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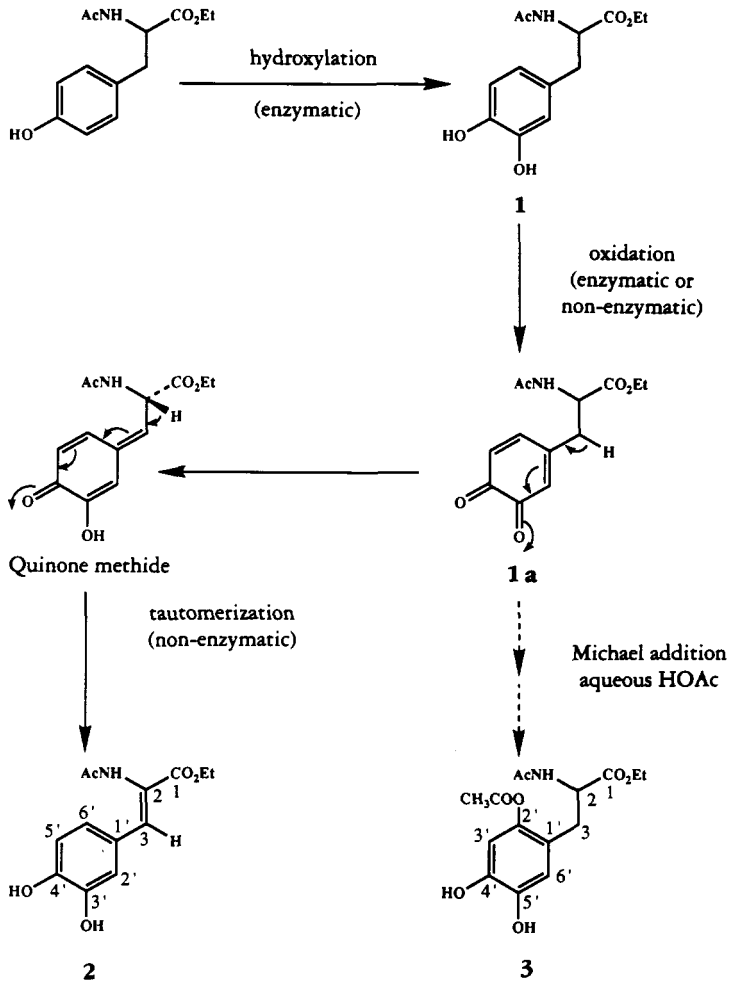


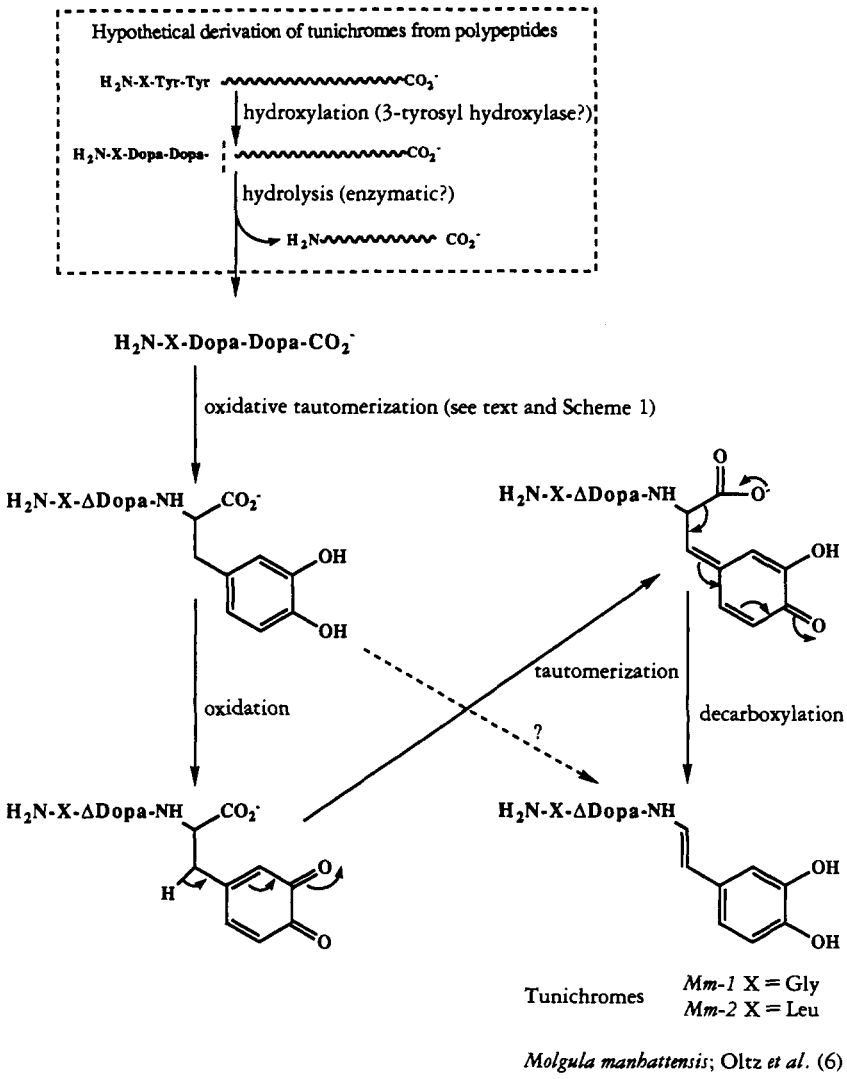
SCHEME 1

mmu −0.7) was consistent with the addition of acetate to **1a**. The ^{1}H-nmr spectrum of **3** lacked the signal expected from H-6′ (numbering as for **2**) and displayed two aromatic proton signals (6.61, s, 1H; 6.43, s, 1H) and an acetoxyl group (1.80, s, 3H). ^{13}C nmr displayed three oxygen-substituted quaternary sp^2 carbons (144.3, s; 142.8, s; 140.9, s), and the chemical shifts were more consistent with a 2-acetoxy-4,5-dihydroxyphenyl group than the 3-acetoxy-4,5-dihydroxyphenyl isomer. Adduct **3** presumably arose from the adventitious addition of acetate during the concentration step, because **3** could not be detected by hplc prior to that step. Michael addition of nucleophiles, such as thiols, amines, and phenols, to quinones is commonplace (19) but addition of carboxyls is exceptional, reported to occur either in highly concentrated HOAc (20) or when a carboxyl moiety is ideally placed sterically for intramolecular ring addition in a mono-substituted quinone (21). The biological significance of compound **3** synthesis is as yet uncertain, but it emphasizes the stringent requirements for carboxyl addition to quinones where such addition is postulated as a sclerotization reaction (22).

Enamide **2** was characterized spectroscopically. The uv spectrum (13) (λ 322 nm, ϵ 14327 $M^{-1}\cdot cm^{-1}$) and chemical reactivities (15–18) of **2** were characteristic of a conjugated *o*-diphenol

with a single α,β-unsaturated substituent. High resolution fabms provided the molecular formula of **2**, $C_{13}H_{15}NO_5$. The ^1H- and ^{13}C-nmr spectra of **2** were compatible with the structure shown and with those of the natural oligopeptides (1–8). Two-dimensional nOe spectroscopy (NOESY) (23) showed correlations between H-2′/H-6′ and NH which confirmed the *Z* geometry of the olefinic double bond. This is the preferred geometry of internal Δ-dopa and Δ-topa in natural and synthetic oligopeptides (1–7). In contrast, the decarboxylated C-terminal residues are *E* geometrical isomers (1–7). [The halocyamines are exceptions to this trend (8).] Enamide **2** also formed upon oxidation of **1** by sodium periodate, showing that tautomerization of *o*-quinone **1a**, detected spectroscopically (uv λ 392, ε 1188) as a reaction intermediate, was independent of the enzyme. Recovery of **2** from *N*-acetyltyrosine ethyl ester was low but increased to 15–25% when **1** was used as the precursor.

The facile tautomerization, under



SCHEME 2

mild conditions in biomimetic buffers, of analogues of peptidyl dopa quinone or quinone methide to give *Z*-Δ-dopa might explain the unusual in vivo unsaturation of dopa and topa in polyphenolic oligopeptides. Previous workers have speculated that tunichromes may be biosynthesized by sequential hydroxylations of tripeptide precursors (6) and that halocyamines from the stolidobranch ascidian *Halocynthia roretzi* are derived from other dopa-containing peptides (8), perhaps similar to unusual dopa proteins recently isolated from the same organism (24). It seems reasonable that such precursors might resemble the dopa scleroproteins found in diverse marine and other invertebrates (9–11). The primary sequence of these proteins is comprised of many oligopeptide tandem repeats. In Scheme 2, we present one of several possible sequences of events illustrating how tunichromes *Mm-1* and *Mm-2* from the tunicate *Molgula manhattensis* (6) [an iron-accumulating stolidobranch relative of *Pyura stolonifera*, source of the iron binding dopa protein ferreascidin (25)] might originate from a polypeptide precursor. Intriguingly, the N-terminus of ferreascidin has the sequence Leu-Dopa-X (X an unknown amino acid), reminiscent of tunichrome *Mm-2* [see Scheme 2 and Oltz *et al.* (6)] (J.H. Waite and M. Wynne, unpublished data). Provided sufficient quantities of proton-accepting anions are present [which is plausible in the unusual ionic environment of tunicate blood cells (26,27)], Δ-dopa and Δ-topa formation should spontaneously follow enzymatic oxidation. Polyphenoloxidase, which oxidizes catechols to quinones, has been detected in ascidian tunics (28). Because the model compound **2** exclusively adopts the *Z* configuration, as do the natural oligopeptides, it seems presently unnecessary to hypothesize any enzymatic requirement for tautomerization of peptidyl dopa quinone in vivo (Scheme 1).

In Scheme 2, we also note that, following enzymatic hydrolysis, a peptidyl C-terminal dopa quinone residue might spontaneously yield decarboxy-Δ-dopa via a quinone methide intermediate in a reaction analogous to that proposed for the oxidative decarboxylation of 3,4-dihydroxymandelic acid (29,30). However, the invariable presence, to date, of diverse aromatic $\Delta^{2,3}$-unsaturated C-terminal residues (i.e., derivatives of dopa, tyrosine, or 6-bromotryptophan) in these oligopeptides (1–8) is consistent with a unitary biosynthetic mechanism, and thus other more complex enymatic or non-enzymatic mechanisms cannot yet be ruled out. For example, pyridine/Cu and quinoline/Cu complexes stereospecifically decarboxylate aromatic (*Z*)-*N*-acyldehydroamino acids to (*Z*)- or (*E*)-enamides, respectively (33).

EXPERIMENTAL

N-AcetylDOPA ethyl ester and its quinone were synthesized by incubating (1 h, 25° in 0.1 M NaH_2PO_4, pH 6.7, with vigorous aeration) 1 mM *N*-acetyltyrosine ethyl ester with mushroom tyrosinase (0.04–0.1 mg/ml), exploiting the 3-tyrosyl hydroxylase and polyphenoloxidase activities of this enzyme (13). Both substrate and enzyme were obtained from Sigma Chemical Co., St. Louis, MO. Rearrangement of the quinone to the unsaturated *o*-diphenol tautomer required the presence of phosphate or other suitable anion (≥0.1 M). Reactions were terminated by addition of glacial HOAc to 4.4 M, and products and residual reactants were concentrated by rotary evaporation at 50°. Components **1**, **2**, and **3** were resolved from byproducts by chromatography on Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA) in 0.2 M HOAc and were further purified to homogeneity by reversed-phase hplc on Microsorb C_{18} columns using 15–30% gradients of MeCN in H_2O, both solvents containing 0.1% trifluoroacetic acid. Catecholic products were identified by uv absolute and difference spectroscopy in HCl and alkaline borate buffers (14), and were chemically characterized by catechol-specific nitration (15–17) and addition (18) reactions. The identities of compounds **2** and **3** were confirmed by fabms, Ft-ir, and nmr spectroscopy. Amino acid hydrolyses were effected in 5 N HCl incorporating 8% phenol and 8% trifluoroacetic acid, modified from Tsugita *et al.* (32).

Compound **2**.—Hrfabms *m/z* 266.1032 (calcd for $C_{13}H_{15}NO_5+H$, 266.1028), Δmmu = −0.4; uv (0.2 N HCl) λ 322 nm (ε = 14327 $M^{-1}\cdot cm^{-1}$);

Ft-ir (film) 3253 (br), 1666, 1597, 1513, 1443, 1371, 1245, 1110, 1025, 868, 812 cm^{-1}; ^1H nmr (300 MHz, Me_2SO-d_6) 9.38 (s, NH, OH peaks coalesce as a broad band), 7.15 (d, $J=1.7$ Hz, H-2′), 7.05 (s, H-3), 6.93 (dd, $J=1.7$, 8.2 Hz, H-6′), 6.75 (d, $J=8.2$ Hz, H-5′), 4.13 (q, $J=7.1$ Hz, 2H, OCH_2Me), 2.00 (s, 3H, *N*-acetyl Me), 1.21 (t, $J=7.1$ Hz, 3H, OCH_2Me); ^{13}C nmr (50.3 MHz, Me_2SO-d_6) 169.3 (s), 165.3 (s), 147.4 (s), 145.1 (s), 132.7 (d), 124.7 (s), 123.4 (s), 123.2 (d), 116.7 (d), 115.5 (d), 60.5 (t, OCH_2Me), 22.5 (q, *N*-acetyl Me), 14.2 (q, OCH_2Me). Multiplicities of ^{13}C signals were deduced from DEPT spectra (31).

Compound **3**.—Hr fabms *m/z* 326.1246 (calcd for $C_{15}H_{19}NO_7+H$, 326.1239), Δmmu $=-0.7$; uv (0.2N HCl) λ 282 nm; ^1H nmr (300 MHz, Me_2SO-d_6) 9.10 (s, OH), 8.85 (s, OH), 8.25 (d, $J=7.7$ Hz, NH), 6.61 (s, 1H, H-6′), 6.43 (s, 1H, H-3′), 4.25 (m, 1H, H-2), 4.03 (q, $J=7.1$ Hz, 2H, OCH_2Me), 2.70 (dd, $J=6.2$, 14 Hz, 1H, $H\text{-}3_b$), 2.54 ($J=8.5$ Hz, $H\text{-}3_a$, multiplicity partially obscured by Me_2SO), 2.23 (s, 3H, *N*-acetyl Me), 1.80 (s, 3H, OCH_2Me), 1.11 (t, 3H, $J=7.1$ Hz, Me); ^{13}C nmr (50.3 MHz, Me_2SO-d_6) 171.7, 169.29, 169.25, 144.3, 142.8, 140.9, 119.0, 116.9, 109.8, 60.4, 52.8, 31.3, 22.3, 20.5, 13.9.

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