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Catecholamine-Protein Conjugates: Isolation of an Adduct of N-Acetylhistidine to the Side Chain of N-Acetyldopamine from an Insect-Enzyme Catalyzed Reaction

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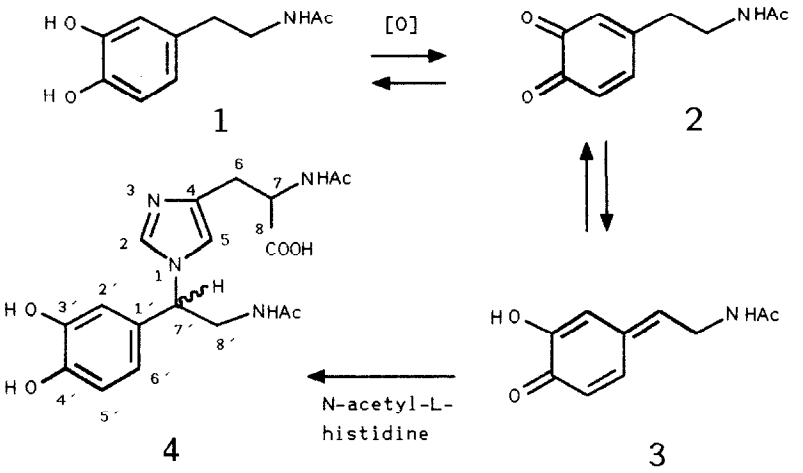
Abstract - N^{α}-Acetyl-N^{τ}-[1-(3,4-dihydroxyphenyl)-2-N-acetylamino-ethyl]-histidine (**4**) was isolated from a biomimetic reaction containing N-acetyldopamine **1**, N^{α}-acetyl-L-histidine and cuticle of silkmoth larvae as an enzyme source. The formation of **4** suggests an 1,6-addition of the N^{τ} imidazole nitrogen to the *p*-quinone methide **3**.

A considerable number of functionally important biopolymers are composed of phenolic subunits, as is commonly known for the structurally ill defined melanins, lignin, and vegetable tannins. Usually, these polymers are conjugated to proteins or polysaccharides.[1] In insects, simple N-acylcatecholamines serve to harden the exoskeleton by a sequence of reactions starting with the oxidation of **1** to the corresponding *o*-quinone[2] **2** which is thought to effect crosslinking and chemical modification of structural proteins.[1,3] More recently, it was discovered that **2** may rearrange to *p*-quinone methide[4] **3** in an enzyme catalyzed reaction.[5,6] We have now isolated adduct **4** from oxidation of **1** in the presence of N^{α}-acetyl-L-histidine under biomimetic conditions, indicating for the first time that analogous catecholamine protein conjugates may indeed occur in nature.

Chromatographic resolution of a reaction mixture[7] containing equimolar amounts of **1** and N^{α}-acetyl-L-histidine in addition to insect cuticle as an enzyme source yields a colourless product [λ_{max}= 278 nm (H_2O/MeOH)]. Plasma desorption mass spectroscopy (PD-MS)[9] reveals the protonated molecular ion at *m/z* = 391.7, confirmed by FAB-MS[11] (*m/z* = 391.2). In PD-MS, peaks at



m/z 194.7 and 198.8 correspond to the N-acetyldopamine and N-acetylhistidine fragments, respectively. Esterification by means of methanol/acetylchloride leads to a derivative which shows fragment ions at *m/z* = 194.7 and 212.8, confirming the presence of the carboxylic group in the histidine derived moiety.

The NMR spectra[12] (see table) clearly indicate, by duplication of most signals, that **4** is in fact a mixture of diastero-isomers. The pattern of aromatic protons shows the presence of a 4-alkyl catechol subunit. CH correlation reveals that H-7′ (δ = 5.148 ppm) is directly connected to C-7′ (δ = 59.46/59.41 ppm), whereas H^{α} (δ = 4.39/4.37 ppm) and C^{α} (δ = 52.19/52.08 ppm) of the histidine moiety appear at the expected shift values. Substitution at N^{τ} of the histidine imidazole is evident from the magnitude of the cross-ring coupling constant ($^4J_{2,5}$= 1.2 Hz).[13,14] The adduct **4** is optically active ($[\alpha]_D^{22}$ 29.2 (c=0.5 in H_2O)). However, partial racemisation at C-α of the histidine residue cannot be exluded with safety.

It is well known that various nucleophiles add readily to either C-5 or C-6 of 4-alkyl *o*-quinones (for review, see[1]). Investigation of sclerotized insect cuticle by solid state NMR[15] has revealed linkages between imidazole nitrogen of histidine residues and ring postions of unidentified metabolites of catecholamines of type **1**.[16] Nonstereoselective 1,6-addition of water[4] or methanol[17] to enzymatically generated *p*-quinone methide **3** have been reported previously. Similarly, nitrogen addition of L-kynurenine to the *p*-quinone methide of N-β-alanyl dopamine affords a diastereoisomeric mixture of papiliochrome II, the yellow wing pigment of papilionid butterflies.[18] The present results provide the first example for the addition of a proteineous

amino acid residue to the *p*-quinone methide of a catecholamine in an oxidative enzymatic reaction. We believe that analogous products may arise not only in insects, but also in other organisms during catecholamine metabolism. Thus, compounds of type **4** may be of more widespread, hitherto unrecognized occurrence in nature.

Table: ^1H and ^{13}C NMR ([D_6]DMSO) data of **4**.

^1H NMR, δ =		^{13}C NMR, δ =	
8.04-7.97	(m, 2 H, NH)		
7.675, 7.669	(2d, $J_{2,5}$= 1.2 Hz, 1 H, H-2)	173.35, 172.11	(C-8)
6.947, 6.936	(2d, $J_{5,2}$= 1.2 Hz, 1 H, H-5)	169.78, 169.73,	(NCO)
6.681, 6.672	(2d, J_o= 8.2 Hz, 1 H, H-5′)	169.22, 169.19	(NCO)
6.646, 6.640	(2d, J_m= 2.4 Hz, 1 H, H-2′)	145.32, 145.19	(C-3′,4′)
6.541, 6.533	(2dd, J_o= 8.2 Hz, J_m= 1.2 Hz,	137.11, 137.00	(C-4)
	1 H, H-6′)	136.04, 135.95	(C-2)
5.148	(br. t, J = 7.4 Hz, 1 H, H-7′)	130.0	(C-1′)
4.39, 4.37	(2dt, $J_{7,6}$= 8.4 Hz, $J_{7,NH}$=	117.57, 115.53,	
	5.2 Hz, 1 H, H-7)	114.33	(C-2′,5′,6′)
3.65	(m, 2 H, H-8′)	115.58, 115.42	(C-5)
2.873, 2.859	(2dd, J_{gem}= 14.8 Hz, J_{vic}=	59.46, 59.41	(C-7′)
	8.6 Hz, 1 H, H-6a)	52.19, 52.08	(C-7)
2.735, 2.728	(2ddd, J_{gem}= 14.8 Hz, J_{vic}= 8.6	42.99	(C-8′)
	Hz, $J_{6,5}$ ca. 0.5 Hz, 1 H, H-6b)	22.47	(CH_3)
1.783, 1.781	(2s, 3 H, $NCOCH_3$)	22.42	(CH_3)
1.759, 1.755	(2s, 3 H, $NCOCH_3$)		

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7 Cleaned cuticle (500 mg) from fifth instar larvae of the giant silkmoth *Hyalophora cecropia* was added to a mixture of 1 (98 mg, 0.5 mmol) and N^{α}-acetyl-L-histidine (99 mg, 0.5 mmol) in 50 ml sodium phosphate buffer pH 7.0 and the mixture was gently shaken for 16 h at 40°C. Decantation was follwed by acidification to pH 3 with formic acid. The solution was then pumped onto Bio-Beads SM-16 (Bio-Rad; 1.6X15 cm column; equilibrated with 0.2 M acetic acid). The adsorbed material was eluted by means of a linear gradient of 0->100% methanol. Fractions showing λ_{max}= 280 nm were combined, evaporated, and the residue subjected to HPLC on Spherisorb ODS 2 (4X250 mm column; elution with a linear

gradient 0->70% methanol in 0.1% trifluoroacetic acid; flow rate: 0.5 ml·min^{-1}).[8] Retention times: N-acetyl noradrenaline[4]: Rt = 8.3 min; **4**: Rt = 10.2 min; **1**: Rt = 12.0 min. Yield of **4**: 15.8 mg (8.1 %).

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[9] BioIon 20 plasma desorption mass spectrometer; the sample was applied from a 0.1% trifluoroacetic acid solution to a nitrocellulose covered target[10]; 10^6 start events.

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[11] Kratos MS 50; matrix: *m*-nitrobenzyl alcohol.

[12] Bruker WM 200; ^{1}H NMR: 200 MHz, [d_6]-DMSO; internal standard: TMS = 0 ppm; ^{13}C NMR: 50.6 MHz, [d_6]-DMSO; internal standard: solvent. ^{1}H and ^{13}C NMR spectra recorded in D_2O lead to the same conclusions.

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