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Synthetic Evidence of the Amadori-Type Alkylation of Biogenic Amines by the Neurotoxic Metabolite Dopegal

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Supporting Information

ABSTRACT: The neurotransmitter metabolite 3,4-dihydroxyphenylglycolaldehyde (dopegal) damages neurons and the myocardium by protein cross-linking, resulting in conglomerations and cell death. We investigated this process on a synthetic scale, leading to the discovery of an Amadori-type rearrangement of dopegal in the reaction with several amino acids and neuropeptides. This alkylation also occurs with neurotransmitters, suggesting an influence of dopegal on neurochemical processes. The rearrangement occurs readily under physiological conditions.

Monoamine oxidase (MAO) is known to catalyze the metabolism of the tyrosine-derived neurotransmitters dopamine 1, epinephrine 5, and norepinephrine 6 with the formation of 3,4-dihydroxyphenylacetaldehyde 2 (dopal) and 3,4-dihydroxyphenylglycolaldehyde 7 (dopegal), respectively (Scheme 1). Further metabolic transformation of these highly reactive aldehydes takes place via reducing (AR/ALR) or oxidizing (ALDH) enzymes arriving at the much less harmful alcohols or acids. When these enzymatic detoxification processes become less efficient, the residing catecholaldehydes give rise to unwanted reactions, eventually leading to protein alkylation and cross-linking.1,2 Neurodegenerative diseases such as Parkinson’s or Alzheimer’s diseases, as well as cardiovascular diseases, are associated with the presence of these reactive aldehydes.3 Meanwhile, knowledge about protein alkylation by the dopamine-derived aldehyde dopal (2) is growing, but the exact reaction mechanism of this process is not completely established.4 Oxidation of the catechol part of dopal 2 to a strongly electrophilic ortho-quinone with reactive oxygen species (ROS) likely plays an important role in irreversible protein alkylation.5 In the early stages of protein alkylation, Schiff’s bases 3 are readily formed in the reaction between the aldehyde of dopal and the lysine side chain amine residues (Scheme 1). The imines thus obtained equilibrate to conjugated enamines 4, a type of intermediate that is frequently observed during Pictet–Spengler reactions.6 In general, under aqueous conditions, imine/enamine adducts are not stable and swiftly hydrolyze to the starting compounds. Recently, a successful identification by mass spectroscopy of some of these imine/enamine intermediates derived from dopal (2) and some small amines was reported.6 The properties of dopegal (7) have not been studied in detail due to the limited stability and availability of this highly reactive aldehyde.7 Dopegal (7) contains an additional α-hydroxyl substituent compared to dopal (2), and this hydroxyl group plays an important role in the reactivity. While dopal requires an additional oxidation step of the catechol part to acquire irreversible reactivity with proteins after Schiff’s base formation, the α-hydroxyl substituent in

Scheme 1. Dopal and Dopegal: Formation and Protein Alkylation

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dopegal actually presents a higher oxidation level, giving rise to direct irreversible alkylation of proteins as will be shown (Scheme 1).

The Amadori rearrangement is a well-known process that starts with imine formation in the reaction of primary amino substituents with reducing sugars such as ribose and glucose (Scheme 2). Initially, an imine is formed, which slowly tautomerizes to the enamine and, after reprotonation, relatively stable α-aminoketones are formed. The Amadori rearrangement is most known because this occurs during food preparations at elevated temperatures. Under physiological conditions, this reaction also takes place slowly and leads after further oxidation of these Amadori products to so-called advanced glycation end products (AGEs), which are harmful in many biological systems. Enhanced glucose levels as in diabetes mellitus-2 stimulate the process and result in irreversible damage to tissues and organs.

The responsible sugars contain an α-hydroxyaldehyde part that resembles the glycolaldehyde part of dopegal. Nevertheless, the remarkably high reactivity of dopegal toward amines, and in particular, toward lysine residues in proteins, has never been recognized as an Amadori-type rearrangement. In a recent publication, a fast reaction between enzymatically synthesized dopegal and the therapeutical dipeptide has never been recognized as an Amadori-type rearrangement. This behavior is not unexpected, given the high reactivity of dopegal toward amines, and in particular, toward lysine residues in proteins, which can be of therapeutic interest. To gain more nucleophilic toward dopegal than the target lysine residues in proteins could be of therapeutic interest. To gain further understanding of this neurochemistry, we started a synthetic project by reacting dopegal with biologically relevant primary amines and amino acids such as β-alanine and lysines, as well as dipeptides and a series of more complex biogenic amines.

The production of dopegal (7) by chemical or enzymatic methods is troublesome, and the limited commercial availability hampers extensive synthetic research. Recently, a one-step preparation of the acetal precursor 13 from catechol (11) and glyoxal dimethyl acetal (12) was described, as an intermediate in a short synthesis of hydroxytyrosol (Scheme 3). The authors described a partial hydrolysis of acetal 13 with aqueous acetic acid and demonstrated a clean conversion to hydrated dopegal in D₂O solution (50% conversion after 3 days at 60 °C). We upscaled the alkylation of catechol with glyoxal dimethyl acetal to multigram quantities and obtained dopegal precursor 13 readily in pure form by direct crystallization. Complete hydrolysis of the dimethyl acetal in 13 was effected with 1 equiv of TFA in D₂O, at a concentration of 0.1 M (16 h at 40 °C). Under these conditions, dopegal (7) is almost entirely present in its hydrated form and contains only 5% of the free aldehyde, together with small amounts of its dimer as a complex mixture of several diastereomers. This dimer is formed reversibly, and the ratio of monomer/dimer is concentration-dependent. To obtain dopegal free from water and acid, the aqueous hydrolysis mixture was freeze-dried. However, in the solid residue, we could not detect the free aldehyde either by NMR, both in DMSO and D₂O, or by IR (no carbonyl absorption in the solid state), indicating that dopegal is completely converted to its cyclic dimer upon concentration. In the literature, a comparable dimerization of the phenyl analogue of dopegal, i.e., mandelaldehyde, is described, in support of our observations. These properties are different from previously synthesized dopegal, which was reported to display aldehyde signals in both NMR and IR spectra. In solution, the dimer is in equilibrium with its (hydrated) monomer but still shows the aldehyde-derived reactivity in further chemistry. After storage of the freeze-dried dopegal for several months, analysis by NMR spectroscopy did not show any decomposition.

With dopegal in hand, in multigram amounts in a stable form, the condensation reaction with several amines was conducted (Table 1). These condensation reactions proceeded readily in water under ambient temperatures (rt to 40 °C) and at reasonable rates with reaction times ranging from 4 to 24 h, depending on the basicity and nucleophilicity of the amines. A pH range from 6 to 8 proved to be optimal. Above pH 8, the darkening of the solution became stronger due to side reactions that always occur when working with these types of catechols. Slightly acidic conditions (pH 4–6) were also effective, so the acidic aqueous solutions obtained from the TFA-catalyzed hydrolysis can be used directly for condensation reactions, adding one equivalent of a base resulted in a pH of around 7 (entries 1–7). The condensation products were isolated by direct crystallization from the reaction mixtures or were obtained by trituration or precipitation after evaporation of the reaction solvents.

The α-aminoketones 14–17 derived from βAla, Gly-Gly, Ac-Lys, and Gln, respectively, were obtained in good yields (entries 1–4). It should be noted that proteins that are targeted by dopegal contain lysine-rich areas that can be considerably more nucleophilic than isolated Ac-Lys (entry 3).
Cysteine condenses with dopegal in a different way and produces a diastereomeric mixture of catechol-substituted thiazolidines \(^\text{18}\), which is a well-known reaction with aldehydes (entry 5).\(^{14}\) Although the formation of this type of thiazolidine is reversible, the thiazolidine adducts with dopegal were stable. Carnosine (entry 6) is a naturally occurring dipeptide that is abundant in the brain and displays a variety of positive effects as an antioxidant, and more importantly, as an antiglycant, preventing the formation of AGEs from sugars and protecting against neurodegenerative diseases such as Alzheimer’s disease.\(^{10,11,15}\) Carnosine and the common neurotransmitters GABA and phenethylamine reacted smoothly, providing \(\alpha\)-aminoketones \(^\text{19, 20, 21}\), and \(\alpha\)-aminoketones, respectively, as solids in pure form (entries 6, 7, and 8). The Amadori products derived from the very important neurotransmitters dopamine (entry 9) and serotonin (not shown) were prone to follow-up reactions and could not be obtained in pure form. As can be concluded from the \(^1\)H and \(^{13}\)C NMR spectra, dopamine-derived aminoketone \(^\text{22}\) was obtained in >80% purity. LCMS analysis showed that, most probably, a follow-up Pictet–Spengler reaction of \(^\text{22}\) with dopegal occurs, followed by further oxidation reactions. Both dopamine and serotonin are discriminated from the other neurotransmitters by their highly \(\pi\)-nucleophilic catechol or indole moieties. Also, histamine behaved differently, and although the reaction took place, we could not obtain the Amadori product \(^\text{23}\) in pure form (entry 10). Finally, also of pyridoxamine, which is under investigation for the depletion of glucose in diabetes mellitus, the Amadori product \(^\text{24}\) was isolated in pure form, albeit in a low yield (entry 11).\(^{15}\)

Because the aromatic ring of the catechol part within the condensation products is conjugated with the carbonyl group, the stability of the catechol part is strongly increased toward oxidation as compared to precursors such as dopamine, dopal, and dopegal. Some reactivity still remains in the \(\alpha\)-aminoketones as condensation in methanol with phenyl acetaldehydes readily yields pyrroles.\(^{16,17}\) A representative example of the reactivity of the catecholaldehydes is the condensation of dopamine-derived aminoketone \(^\text{22}\) with dopal \((\text{1:1 ratio})\) at a neutral pH to give pyrrole \(^\text{25}\) (43% yield) without the use of oxidants and or catalysts (Scheme 4). The isolation and characterization of pure pyrrole \(^\text{25}\) is a further structure proof of the starting aminoketone \(^\text{22}\) (vide supra). Pyrrole \(^\text{25}\) could also be obtained by a one-pot, two-step condensation of dopegal and dopal\(^{18}\) with dopamine in a 1:1:1 ratio, at a neutral pH, albeit in a lower yield. This type of bis-aryl pyrroles and their further oxidized metabolites are well-known bioactive natural products, e.g., the lamellarines and ningaline.\(^{19}\) Bax et al. described an interesting, related pyrrole-forming reaction, whereby 2 equiv of dopal react with Ac-Lys under oxidative conditions, suggesting a mechanism for cross-linking processes in AD-related synuclein aggregation.\(^{4c,d,5}\)

In summary, we have unraveled an efficient amine alkylation process with the endogenous neurotoxin dopegal in the absence of an external oxidant. This Amadori-type rearrangement, which is actually an intramolecular redox reaction, is much faster than the natural Amadori reaction of amino...
residues in peptides with sugars. The resulting catechol-
aminoketones are not known in the literature, and further
(oxidative) conversion into harmful AGE-like derivatives
cannot be ruled out. The potential value for these compounds
can be found in the application as biomarkers for the
accumulation of dopegal in biological systems. Finally, our
scalable synthesis of dopegal enables its further use in
neurobiochemical studies, which will be carried out in due
course.

**EXPERIMENTAL SECTION**

**General Information.** All reactions concerning catechol deriva-
tives were performed with degassed solvents and under argon to
minimize darkening. Reagents and precursors were purchased with
the highest purity (usually >98%) from Sigma-Aldrich and
Fluorochem and used as received. Reactions were monitored by
thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck
silica gel plates (60F-254). SilaFlash P60 (particle size 40–63 μm)
was used for silica column chromatography. NMR spectra were
recorded on Bruker DRX-500, -400, and -300 MHz instruments and
calibrated on residual undeuterated solvent signals as an internal
standard. High-resolution mass spectra (HRMS) were recorded with a
TOF mass spectrometer of the type AccuTOF GC v 4g, standard. High-resolution mass spectra (HRMS) were recorded with a
Bruker Alpha FTIR
ionization method. IR spectra were recorded on a Bruker Alpha FTIR

**Synthetic Procedures.** 2-(3,4-Dihydroxyphenyl)acetaldehyde (Z, Dopal). KOTBu (1.35 g, 12.0 mmol) was added in one portion to a stirred suspension of (methylthiomethyl)triphenylphosphonium chloride (4.12 g, 12.0 mmol) in THF (30 mL) at 0 °C. After 20 min, a solution of 3,4-bis((tetrahydro-2-
chloride (4.12 g, 12.0 mmol) in THF (30 mL) at 0
°C. After 20 min, (3,4-Dihydroxyphenyl)-2-oxoethyl)glycylglycine (Gly-Gly). 3-(((2-(3,4-Dihydroxyphenyl)-2-oxoethyl)amino)propanoic Acid

**Note**

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N'-Acetyl-N-(2-(3,4-dihydroxyphenyl)-2-oxoethyl)-l-lysine (16) from Ac-Lys. Dopeg 7 (75.6 mg, 0.45 mmol) was added to a solution of N'-acetyl-lysine (75.2 mg, 0.40 mmol) in water (1.5 mL), and the resulting solution was stirred at 40 °C for 18 h. Evaporation of the solvent and trituration of the residue with methanol (3 mL) at 50 °C gave 16 as a beige solid (75.0 mg, 0.222 mmol, 55%); mp 194–197 °C; IR 3500–2500, 1662, 1585 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.80 (d, J = 7.2 Hz, 1H), 7.37 (m, 2H), 6.85 (d, J = 8.1 Hz, 1H), 4.32 (s, 2H), 4.07 (m, 1H), 2.73 (t, J = 7.5 Hz, 2H), 1.84 (s, 3H), 1.77–1.43 (m, 4H), 1.43–1.39 (m, 2H); ¹³C{¹H} NMR (126 MHz, DMSO-d₆) δ 194.15, 174.7, 169.3, 152.5, 146.2, 127.1, 121.1, 116.0, 115.5, 53.9, 53.2, 48.6, 32.0, 28.1, 25.9, 23.4, 23.0; HRMS (FD⁻) m/z calcd for C₁₇H₁₉N₄O₆ [M⁺H⁺] 397.1456, found 397.1457.

(2-(3,4-Dihydroxyphenyl)-2-oxoethyl)-l-glutamine (17) from Gln. Dopeg 7 (75.6 mg, 0.45 mmol) was added to a solution of glutamine (58.4 mg, 0.40 mmol) in water (1 mL), and the resulting solution was stirred at 40 °C for 18 h. After cooling, the precipitated product was filtered and washed with water (0.5 mL), isopropanol, and ether. The filtrate was concentrated to obtain a second batch of off-white product 17 (85.4 mg, 0.289 mmol, 72%); mp 151.6 mg, 0.2 mmol) in methanol (2 mL) was added to a solution of glutamine (58.4 mg, 0.40 mmol) in water (1 mL) and methanol (1 mL) was purged with argon for 30 min. Sodium hydrogen carbonate (84.0 mg, 1.0 mmol) was added; degassing was continued for 10 min. The reaction flask was wrapped in aluminum foil, and the reaction mixture was stirred at rt for 72 h. The precipitate was collected, washed with water (3 times) and a 1/1 mixture of isopropanol and diethyl ether. The brown product 21 (157.6 mg, 0.521 mmol, 65%) was stored at 4 °C; mp 183–192 °C; IR 3500–2500, 1645, 1589 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.79–7.73 (m, 1H), 7.41–7.40 (m, 1H), 4.06 (s, 2H), 3.86–3.67 (m, 2H), 3.25 (t, J = 7.2 Hz, 1H), 2.38 (t, J = 6.5 Hz, 2H), 2.29 (s, 3H), 1.30 (dd, J = 10.5, 6.1 Hz, 1H); ¹³C{¹H} NMR (126 MHz, DMSO-d₆) δ 196.0, 179.4, 172.6, 151.5, 149.0, 146.2, 126.1, 121.6, 115.8, 61.0, 52.1, 31.9, 26.9; HRMS (FD⁻) m/z calcd for C₁₇H₁₉N₄O₆ [M⁺H⁺] 341.1180, found 341.1182.

1-(3,4-Dihydroxyphenyl)-2-(phenethylamino)ethan-1-one (21) from Phenethy lamine. A solution of phenethyamine (484 mg, 4.0 mmol) in methanol (2 mL) was mixed with dopeg 7 (4 mL of a 0.1 M solution in 0.1 M TFA, 0.40 mmol) and stirred at 45 °C overnight. Evaporation of the solvents and triturating with isopropanol gave 21 (TFA-salt, 0.266 mmol, 66%); mp 179–181 °C; IR 3769, 1676, 1669 1605 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 10.24 (broad, 1H), 9.58 (broad, 1H), 9.02 (broad, 2H), 7.40–7.34 (m, 4H), 7.29 (m, 3H), 6.91 (d, J = 8.2 Hz, 1H), 4.70 (s, 2H), 3.22 (t, J = 8.3 Hz, 2H), 3.09–2.93 (m, 2H); ¹³C{¹H} NMR (126 MHz, DMSO-d₆) δ 190.0, 158.9, 158.7, 152.8, 146.1, 137.6, 129.1, 129.1, 127.3, 125.9, 122.2, 115.8, 115.2, 52.2, 48.3, 31.9; HRMS (FD⁻) m/z calcd for C₁₈H₁₈N₂O₆ [M⁺] 272.1281, found 272.1278.

Note
was stirred in the dark for 42 h under O₂-free conditions. Evaporation and flash chromatography (DCM/MeOH 90/10 and 87/13) were performed quickly. Product 25 (36.4 mg, 0.0869 mmol, 43%) was obtained after drying (0.1 mbar, 40 °C) as a colorless glass, turning red/brown upon standing. A one-pot reaction of the three components in a 1/1 mixture of methanol and water, with 48 h intervals, gave new components in a 1/1 mixture of methanol and water, with 48 h obtained after drying (0.1 mbar, 40 °C).

Jan Koomen, who sadly passed away on January 16, 2019 as a financial interest.

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The authors declare no competing financial interest.

**REFERENCES**


12. Dopal 2 was prepared by a Wittig reaction/acid hydrolysis route, see Experimental Section.
