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Review

Photochemistry and free radical stabilisation of the captodative centre

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ABSTRACT

The aim of this article is to highlight the importance of the captodative effect in the amino acids and peptides. A few amino acids are mentioned and their crucial biological role is explained. Several dipeptides are cited and their free radical scavenging properties are examined. Also, the importance and utilisation of the captodative centres in amino acids in some photochemical reactions are reviewed.

KEYWORDS: photolysis, captodative centre, free radical scavengers, captopril, dipeptides, neutrophil assay, xanthine/xanthine oxidase

INTRODUCTION

Several years ago we embarked upon a research project aiming to use the principle of the captodative effect: the combined action of an electron-withdrawing (captor) and an electron-releasing (donor) substituent on a radical centre which, leads to an enhanced stabilization of the radical. It is the purpose of this review to shed some light on the concept and to highlight the implication of such phenomena on the biological systems. First, we will describe how the intuitive idea based on the fundamental principles governing the stabilization of radicals and ions led us to formulate the concept. We will then demonstrate that the concept is well founded, by emphasizing the most significant experimental evaluations concerning it and especially by describing the mechanistic consequences for homolytic reactions and its applications in selective organic synthesis.

The importance of the α–amino acids is well known and its biological diversity is equally well established; this attracted many organic chemists for many years [1-5].

Captodative effect

Phenylalanine derivatives are of particular interest due to their medicinal role in compounds such as:

1. L-Dopa

This material has other names such as: (2*S*)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid, L-3,4-dihydroxyphenylalanine, Levodopa, Sinemet, Parcopa, Atamet, Stalevo, Madopar, Prolopa. It is a naturally occurring substance and it is a psychoactive drug found in certain kinds of food and herbs (e.g. *Mucuna pruriens*). It can also be synthesised from the essential amino acid L-tyrosine (TYR) in the mammalian body and brain. L-dopa is the precursor to the neurotransmitters: dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). These are collectively known as “catecholamines”. Apart from its natural and essential biological role, it is also used in the clinical treatment of “Parkinson’s disease” (PD) and “Dopamine-responsive dystonia” (DRD) [6].

Levodopa is used as a prodrug to increase dopamine levels for the treatment of Parkinson's disease, since it is able to cross the blood-brain

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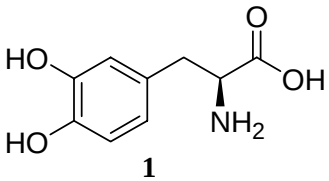
barrier whereas dopamine itself cannot. Once levodopa has entered the central nervous system (CNS), it is metabolized to dopamine by aromatic-L-amino-acid decarboxylase. However, conversion to dopamine also occurs in the peripheral tissues, causing adverse effects and decreasing the available dopamine to the CNS, so it is standard practice to co-administer a peripheral DOPA decarboxylase inhibitor. In work that earned him a Nobel Prize in 2000, Swedish scientist Arvid Carlsson first showed in the 1950s that administering levodopa to animals with Parkinsonian symptoms would cause a reduction of the symptoms. The 2001 Nobel Prize in Chemistry was also related to L-DOPA: the Nobel Committee awarded one-fourth of the prize to William S. Knowles for his work on chirally catalysed hydrogenation reactions, the most noted example of which was used for the synthesis of L-DOPA [7].

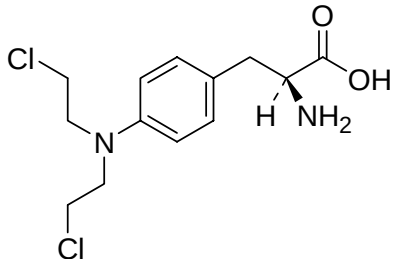


1

(2*S*)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid [L-Dopa].

2. Melphalan

The approach to the treatment of patients with multiple myeloma (MM) include the introduction of the melphalan/prednisone combination in the 1960s; high-dose chemotherapy supported by autologous stem cell transplant (auto-SCT) in the 1980s; and the more recent introduction of the novel agents, thalidomide, lenalidomide, bortezomib, and pegylated liposomal doxorubicin [8]. Results of clinical trials have demonstrated that treatment with thalidomide, lenalidomide, and bortezomib improves overall duration of response, progression free survival (PFS) and overall survival when compared with conventional therapy in patients with newly diagnosed MM (NDMM) or relapsed/refractory MM (RRMM) (reviewed by [9]). Historically, age and eligibility for auto-SCT were the primary basis for treatment selection. Genetic-based assessment is increasingly being used to guide treatment selection, which has demonstrated enhanced efficacy when newer agents are incorporated into the treatment program [10]. This trend reflects an improved understanding of the genetic heterogeneity that marks this complex disease.

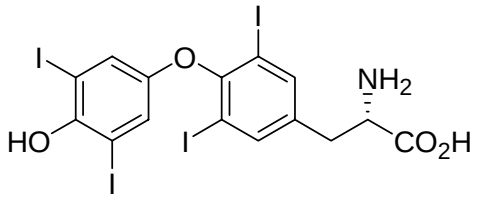


2

(2*S*)-2-amino-3-{4-[bis(2-chloroethyl)amino] phenyl}propanoic acid [Melphalan].

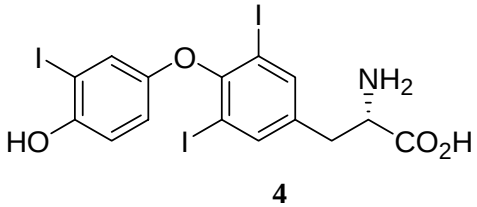
3. Levothyroxin

This is a hormone replacement medicine usually given to patients with thyroid problems, specifically, hypothyroidism. Also, it has been given to people who have “goitre” or enlarged thyroid glands. These compounds are used in the treatment of Parkinson’s disease, cancer, and myxoedema consecutively [11-13].



3

(2*R*)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy) -3,5-diiodophenyl]propanoic acid.



4

(2*R*)-2-amino-3-[4-(4-hydroxy-3-iodophenoxy)-3, 5-diiodophenyl]propanoic acid.

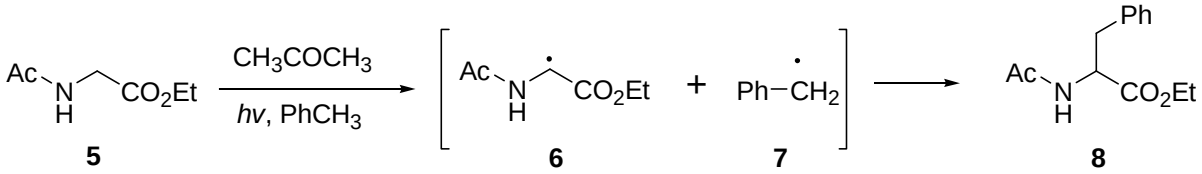
4. Phenylalanine derivatives

These are generally prepared using ionic reactions [14] while alpha-amino acids are commonly prepared using radical carbon-carbon bond-forming reactions. It is surprising that the latter received a lot less attention; bearing in mind the mild (neutral) reaction conditions used and the compatibility of radicals with a wide range of common functional groups which include amides and esters.

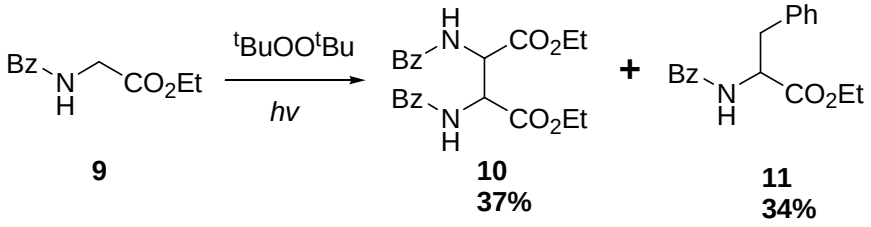
Elad and co-workers [16] reported the synthesis of glycine derivatives employing an intermolecular radical coupling reaction [15] (Scheme 1). They showed that UV irradiation of *N*-acetylglycine ethyl ester **5** in the presence of acetone and excess toluene led to the selective formation of phenylalanine **8**, although the yield was only 3-5%. The authors [16] proposed a mechanism for this reaction which involved coupling of captodative radical **6** with a benzyl radical **7**, these radicals were generated from abstraction of a hydrogen atom from **5** and toluene, in that order, by an excited acetone molecule. This method was extended by the authors [17] to alkylation of glycine residues within peptides and proteins, and also this could be accomplished using visible light, when an α-diketone and *di-tert*-butyl-peroxide were used in place of acetone.

Easton and co-workers [18-19], have reported the formation of butanedioate **10** in 37% yield on UV irradiation of a mixture of glycine **9** and *di-tert*-butyl peroxide (Scheme 2) these radical coupling reactions have been carried out in the absence of an alkylating agent, to form amino acid dimers **10**. In this reaction it is *tert*-butoxyl radical or methyl radical (produced on beta-scission of *t*-BuO$^{\cdot}$), that abstracts the alpha-hydrogen atom from **9**. Although the use of *di-tert*-butyl peroxide, in place of acetone, leads to more efficient hydrogen-atom abstraction, as methyl radicals are produced, a competitive coupling to form alanine **11** (in 34% yield) was also observed. With a view to developing an efficient photochemical approach to phenylalanines; the authors also reported the alkylation of various glycine derivatives using *di-tert*-butyl peroxide (as the initiator) in the presence of substituted toluenes.

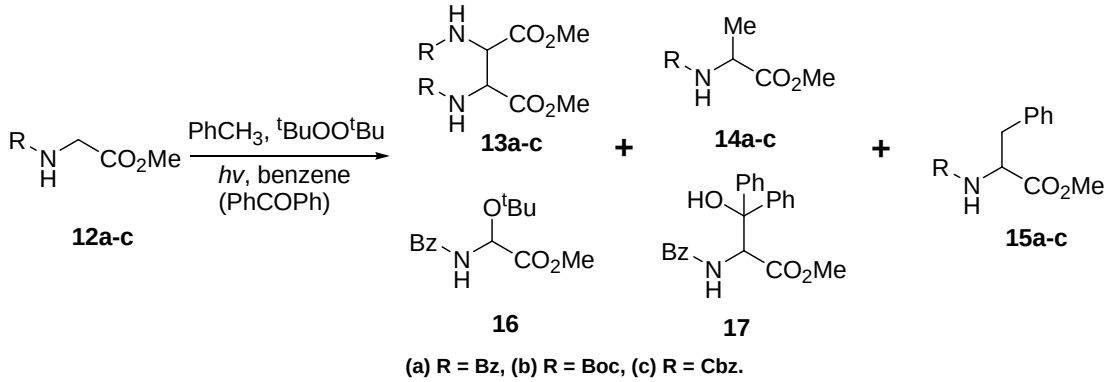
Simon *et al* [2b] investigated the irradiation of **12a-c** in the presence of toluene and *di-tert*- butyl peroxide (Scheme 3). The mixture was irradiated using either a 125 or 400 W UV lamp in degassed benzene and a variety of reactions were carried out using different concentrations of the reagents. In all cases, 1,2-diphenylethane (derived from dimerisation of **7**) and small amounts of butanedioate **13a** and alanine **14a** were isolated, but the major product was the desired



Scheme 1. Irradiation of ethyl (acetylamino) acetate 5 in the presence of toluene and acetone.



Scheme 2. Photolysis of ethyl (benzoylamino)acetate in the presence of *di-tert*-butyl peroxide.



Scheme 3. Photolysis of **12a-c** in the presence of toluene and *di-tert*- butyl peroxide.

phenylalanine **15a**. The reaction was most selective for **15a** when a 1:2:5 ratio of **12a**: peroxide: toluene was used; under these conditions **15a** was formed in 27% yield, or 59% yield based on recovered **12a**. Similar results were obtained using *N*-Boc and *N*-Cbz protected glycines **12b** and **12c**, to form **15b** and **15c**, respectively. The selective alkylation of **12c** is particularly interesting because (competitive) hydrogen-atom abstraction at the benzylic position of the Cbz-protecting group is also possible although no products derived from this reaction were isolated. In an attempt to decreasing the reaction time, the authors performed the alkylation of **13a** using a 400 W UV lamp and although the total yield of products increased (from 70 to 91%), an additional product, namely α-*tert*-butoxy-glycine **16**, was isolated in 13% yield. It was expected that the formation of **16** presumably reflects a higher concentration of the $^{t}BuO^{\cdot}$ radical (which couples with the captodative radical) when using a more powerful lamp, but the yield of **16** was minimised by increasing the concentration of **12a-c** [29(54)%]. Further experiments investigated the addition of photosensitisers and when benzophenone was used in combination with 10 equivalents of toluene an optimum yield of 37% (or 57% based on recovered **12a**) was isolated for **15a**. The authors also investigated the use of benzophenone which also produced minor amounts of the serine derivative **17**, derived from coupling of the captodative radical with $Ph_2(HO)C^{\cdot}$. When the same photolysis was carried out in the absence of toluene, **17** was isolated in 23% yield (or 50% based on recovered **12a**).

Demiryurek *et al* [20] reported the synthesis, characterisation and the study of 67 compounds. These were cyclic and linear dipeptides. Some examples are illustrated in Tables 1-3.

Phorbol myristate (PMA) and ionomycin chemiluminescence (Neutrophil Assay)

Demiryurek *et al* [20] used the following method to perform the neutrophil assay: Stock leukocyte cell suspension (0.45 mL) was diluted with (0.45 mL) phosphate buffered saline (PBS, 10 mM KH_2PO_4 and NaCl, pH 7.4) in a cuvette containing a stirring bar. Following preincubation at 36^{o}C for 5 minutes, the cuvette was transferred to measuring chamber (37^{o}C) and (0.1 mL) luminal (225 µM; final cuvette concentration) was added, producing a final cell yield of $4.5x10^{6}$ cells/mL. Then stimulant (PMA or ionomycin) was added to yield final cuvette concentrations of 0.8 µM and 3 µM respectively. Chemiluminescence from essentially the neutrophil fraction of the leukocytes was measured continuously for 15 minutes. Approximately 30 aliquots were obtained per (10 mL) of blood. The results are shown in Tables 1-3.

X-XO-induced chemiluminescence

In order to characterise the X-XO induced chemiluminescence, 0.9 mL phosphate buffered saline was mixed with 0.1 mL luminal (225 µM; final cuvette concentration) in a cuvette containing a stirring bar. Following the addition of 10 µL xanthine (final concentration 10^{-4}M), and 6.25 µL xanthine oxidase (final concentration 2.5 mU/m) to the cuvette, the chemiluminescence produced

Table 1. Diprotected dipeptides.

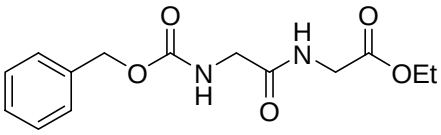
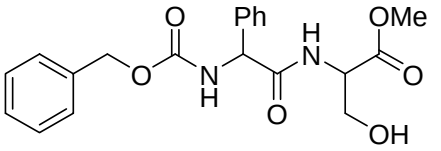
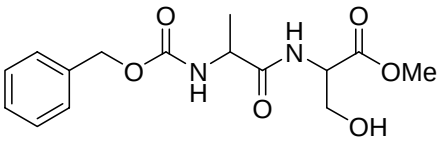
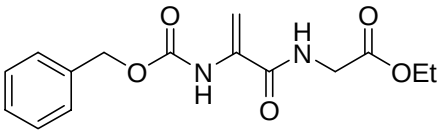
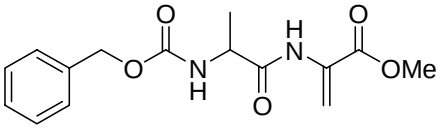
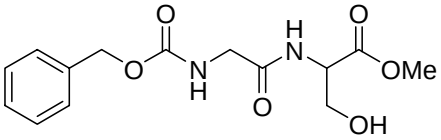
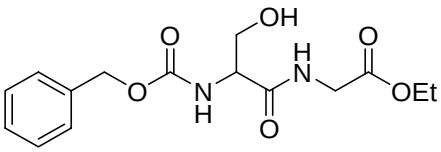
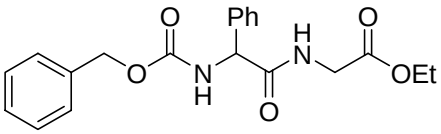
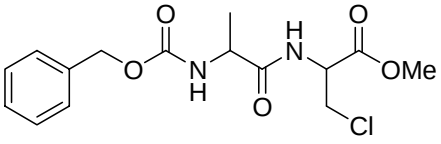
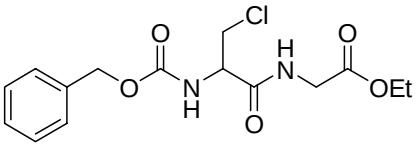
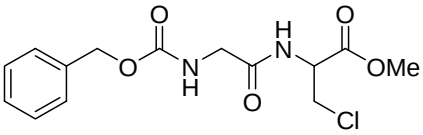
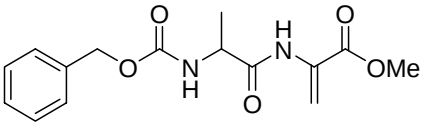
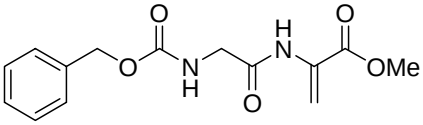
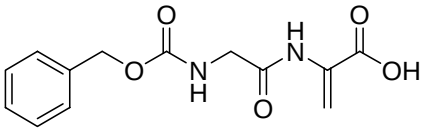
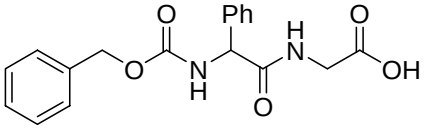
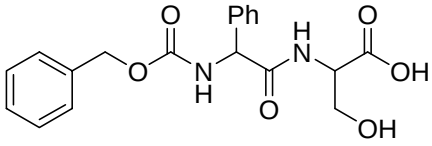
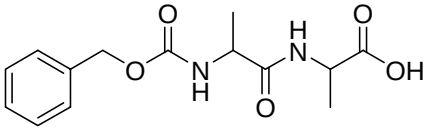
	Substance	Neutrophil assay# -/+%	X-XO-/+%	References
18		-14 (10^{-3}) 0 (10^{-4})	-34 (10^{-3}) 8 (10^{-4})	[20], [21]
19	 DL,DL	-34^{+} (10^{-3}) -12 (10^{-4}) 8 (10^{-6})	-32 (10^{-3}) -18 (10^{-4}) 6 (10^{-6})	[20]
20	 DL,DL	-21 (10^{-3}) ---------- -8 (10^{-6})	-26 (10^{-3}) -23 (10^{-4}) -3 (10^{-6})	[20]
21		-28 (10^{-3}) ---------	-4 (10^{-3}) 27 (10^{-4})	[20], [22]
22	 DL	-54 (10^{-3}) -37 (10^{-4}) -27 (10^{-6})	-22 (10^{-3}) -19 (10^{-4}) -2 (10^{-6})	[20]
23	 L	N/T	N/T	[20]
24	 L	N/T	N/T	[20]
25	 DL	N/T	N/T	[20]
26	 DL,DL	N/T	N/T	[20]

Table 1 continued..

27	 L	N/T	N/T	[20]
28	 L	N/T	N/T	[20]
29	 DL	N/T	N/T	[20]
30		N/T	N/T	[20]

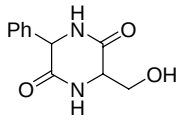
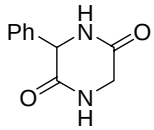
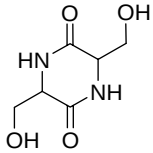
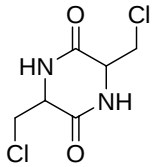
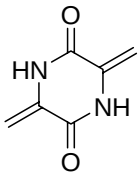
#Cl_{max} values

Table 2. Monoprotected dipeptides.

	Substance	Neutrophil assay# -/+%	X-XO -/+%	References
31		-28 (10^{-4})* -16 (10^{-6})	-35 (10^{-4}) 14 (10^{-6})	[20]
32	 DL	-32 (10^{-4}) ----------	-14 (10^{-4}) ----------	[20], [24]
33	 DL,DL	-4 (10^{-4}) -2 (10^{-6})	-4 (10^{-4}) ----------	[20]
34	 DL,DL	-10 (10^{-4}) 0 (10^{-6})	-19 (10^{-4}) ----------	[20], [23]

#Cl_{max} values
*M concentration is shown in ( ).

Table 3. Cyclic dipeptides.

		Neutrophil assay#-/+%	X-XO -/+%	References
35	 DL	-5 (10^{-3})***** ----------	-12 (10^{-3}) -1 (10^{-4})	[20]
36	 DL	-12 (10^{-3}) -9 (10^{-4}) 1 (10^{-6})	-1 (10^{-3}) -------- --------	[20], [25]
37	 DL,DL	N/T	N/T	[20], [26]
38	 DL,DL	N/T	N/T	[20]
39		-57^{+} (10^{-3})***** -25 (10^{-4}) 3 (10^{-6})	-49 (10^{-3}) -18 (10^{-4}) 29 (10^{-4})	[20]

#Cl_{max} values,
*****M concentration is shown in ( ).
+The net effect, i.e. drug-solvent effect is indicated for the neutrophil assay.

Table 4. Model compounds tested.

Substance	Neutrophil Assay#-/+%	X-XO-/+%
Captopril	-12^{+} (10^{-3})***** -6 (10^{-6})	-75 (10^{-3}) -28 (10^{-6})
Glutathione	1 (10^{-3})	-9.8 (10^{-3})
SOD	-87 (500 U/mL)	-98 (10 U/mL)
Catalase	-52 (3000 U/mL)	-97 (1000 U/mL)
L-Ascorbate	-5 (10^{-3})	-100 (10^{-3})
Sodium azide	-86 (10^{-3})	13 (10^{-3})
Mannitol	-14 (10^{-1})	-9 (10^{-1})

#Cl_{max} values
*****M concentration is shown in ().
+The net effect, i.e. drug-solvent effect is indicated for the neutrophil assay.

was measured continuously for 5 minutes. Duplicate assays were performed in all experiments and the results are shown in the above tables.

CONCLUSION

It seems quite apparent that some of the dipeptides have reasonably similar activity to the standard free radical scavenging compounds such as: Captopril, Glutathione, SOD, Catalase, L-Ascorbate, Sodium azide and Mannitol. When comparing the results from Tables 1-3 and Table 4 (i.e. Captopril in the neutrophil assay); a clear picture emerges that some of the liner dipeptides have similar activity: compound **18** in Table 1 has a value of [-14 (10^{-3})] while the Captopril gave [-12 (10^{-3})]. However, X-XO assay for Captopril gave [-75 (10^{-3})] which is slightly different from compound **18** in Table 1 which gave [-34 (10^{-3})]. However, the cyclic ones are more in line with the standard compounds. For example: compound **36** in Table 3 gave a value of [-12 (10^{-3})] in the neutrophil assay while Captopril gave the same value in the same assay. In summary, the authors [20] have succeeded in synthesising a set of compounds which have the same characteristics as the well known free radical scavengers such as Captopril and glutathione.

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