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SYNTHESIS AND CHARACTERIZATION OF α-AMINO-N-PHENYL SUCCINIMIDE AS PRECURSOR FOR CORRESPONDING THIONIMIDES

Ibrahim S Al-Adham[1], Zuhair Muhi-Eldeen[2*], Elham Al-Kaissi[1], Tawfik Arafat[2], Bahaa Shafiq[1]

[1]Depart. of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Petra, Amman, Jordan
[2] Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, University of Petra, Amman, Jordan

***Correspondence for Author: Dr. Zuhair Muhi-Eldeen**
Depart. of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Petra, Amman, Jordan.

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ABSTRACT

The synthesis and structural elucidation of α-amino-N-phenylsuccinimide through the IR, ^{h}NMR, ORD, and elemental analysis was described as precursor to thionimides, L-(S)-aspartic acid served as starting material and was utilized to investigate routes applicable to the synthesis of α-amino-N-phenylsuccinimide of known absolute configuration. Various functional groups such as benzyloxycarbonyl group (cbz), t-butyloxycarbonyl group (BOC) were investigated in the synthesis. α-amino group protection utilizing the t-butyloxycarbonyl function proved most applicable to the synthesis of the desired L-α-amino-N-phenylsuccinimide hydrochloride. The five-step synthesis to the α-amino-N-phenylsuccinimide hydrochloride having the known L-(S) absolute configuration involves reaction of L-aspartic acid with some protecting group Z, followed by anhydride formation, reaction with aniline to form the anilide and subsequently ring closer affording the imide. Hydrolysis under mild acid conditions yielded optically pure L-(S)-α-amino-N-phenylsuccinimide. However, all attempts to convert this compound to the desired thionimide were not successful. The five-step sequence of the reactions involving the described protecting group was studied utilizing optical rotatory dispersion as a mean of detecting racemization. The sign of the Cotton effect for various intermediates in the synthesis of the final imide was correlated with the absolute configuration of the asymmetric center. NMR analysis of the ABX type for alicyclic protons of the imide ring was discussed. In all cases NMR analysis of the relatively flat heterocyclic ring showed cis-coupling (8.74-11.90 Hz) to be larger than trans-coupling (4.74-6.79 Hz). As expected, the germinal coupling for the extended π- system was larger than either cis- or trans-coupling.

KEYWORDS: α-amino-N-phenylsuccinimide, ionizing radiation, Protective agent, Stereochemistry, Optical rotatory dispersion, Alicyclic H-NMR analysis.

INTRODUCTION

Ionizing radiation, irrespective of its origin, has been shown to cause immediate changes in irradiated tissue. Such chemical changes result in metabolic derangements which may lead to cellular damage and eventually to death of the cells and the organism.[1] With this discovery that animals can be partially protected against the deleterious effects of ionizing radiation by prior administration of certain specific chemical compounds, a new field of investigation opened.

Owing to the growing application of nuclear energy for peaceful purposes the study of chemical protection has attracted increasing interest and today this field has resulted in the development of a special branch of biochemical pharmacology and medicinal chemistry.[2-11] The available evidence indicate that chemical protection against ionizing radiation is brought about by a reduction in the immediate chemical and biochemical lesion. The mechanism of chemical protection is therefore intimately connected with the mechanism of radiobiological damage and the problems can be profitably be discussed together. It is therefore necessary to consider briefly some aspects of radio physics, radio chemistry and mammalian radiobiology.

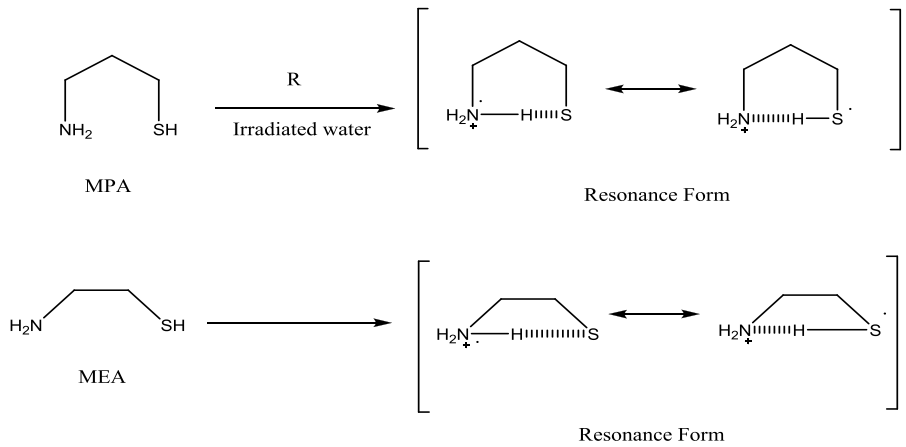
Approximately one-third of the absorbed radiation energy leads to the ejection of an electron from molecules present in the biological system being studied. This results in the formation of free radicals.

In general, free radicals are highly reactive molecules, which have a half-life of only a fraction of second. Most of the radicals formed in irradiated tissues have a very short lifetime, but recent experiments indicate semi-stable radicals are also formed.[12] A free radical produced by ionization of a complex molecule will eventually become stabilized by chemical changes occurring either at the site of the original ionization or after migration of the free radical to a more stabilized position. These chemical changes, caused by direct ionization of molecules in the biological system, are said to be produced by the "direct action of ionizing radiation". Owing to the predominance of water

molecules in soft tissues, a greater portion of the ionizing radiation will absorbed by these molecules. The radiolysis of water[13, 14] results in the formation of molecules H_2O_2, H_2 and a number of highly reactive radicals (H*, HO*, HO_2*, O_2^-, and H_2^+). For many years the radiochemical production of H_2O_2 was assumed to be of major significance, but during the last 10 to 15 years the emphasis has shifted to the importance of radical formation. The above radicals have been found to be very unstable having a life-time in tissues of less than 10^{-4} seconds.[15] The radiolysis of water is dependent upon the presence or absence of molecular oxygen. When oxygen is absent from the solution, X-rays and gamma-rays do not give rise to H_2O_2 and the amount of HO_2* formed is substantially less.[13] The fact that radiobiological damage, in vivo and in vitro, is markedly reduced when the irradiation is carried out under reduced oxygen pressure (the oxygen effect) is mainly attributed to the necessity of paramagnetic O_2 in the radical producing reactions.[16] In tissues, the radicals will eventually react and lead to chemical transformation of the cellular constituents. The biological effect brought about by this mechanism is termed the "indirect action of ionizing radiation".

Research plan

A most important goal of research with chemical protective agents has been to decrease the effect of ionizing radiation on living systems, particularly human beings. A direct approach to this problem would appear to be derivable from studies of radiation effects on vital components the cells. As discussed previously, living systems may be affected by ionizing radiation through a direct or indirect mechanism. Many theories concerning these mechanisms have been proposed. These theories received support from both the biological and chemical points of view and continue as the basis for much experimentation. Free radical formation anoxia mechanisms, metal ion complexing, energy transfer mechanisms, and possibly disulfide formation may all be involved in the activity of the aminothiols. As early as 1955, it was noted that a common feature of relatively good protective agents was an unshared pair of electrons.[17] This observation was used as support for the suggestion of a free radical trapping mechanism. In addition, some of the most protective agents in mammals contain a thiol group. In the aliphatic series, it was noted that those compounds containing only a thiol group and no β or ɣ amino function were considerably less active as previously reported.[18] Interestingly, ɣ -mercaptopropylamine (MPA) is two and one-half times as effective as β-mercaptoethylamine (MEA) in protecting animals against ionizing radiation. Doherty [19, 20] suggested this difference in activity to be due to the greater ability of ɣ -mercaptopropylamine to trap free radicals by forming a more stable six-membered ring intermediate.[21] This intermediate would enable delocalization of the odd electron over three atoms; β-mercaptoethylamine may form a less favorable five-membered ring system in order to obtain the same delocalization.

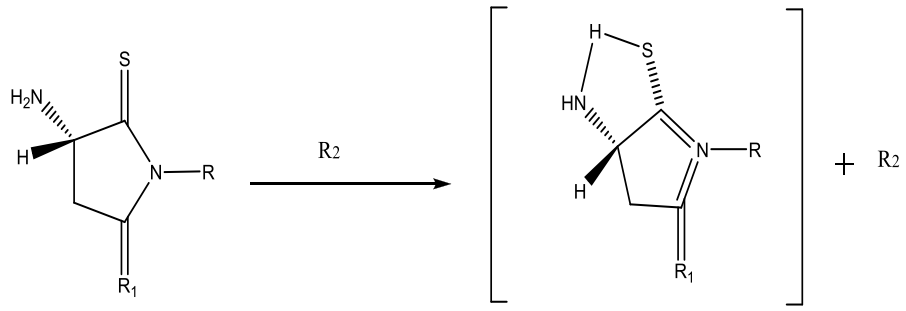


Electronic approach to the design of radioprotective agents

α- aminomonothionsuccinimide (**1**) and α-aminodithionsuccinimide (**2**) are compounds containing a basic amino group which is located two or three carbons from an S atom. This is a relationship not unlike that found in β-mercaptoethylamine and ɣ -mercaptopropylamine respectively.

In these systems, however, the S atom is in the form of a C=S function rather than in the form of a SH group. It seems likely that compounds such as **1** and **2** may readily act as a free radical trapping agents and form species such as **2**.

The imide substituent R may be H, alkyl or aryl. However, investigations with simple thionimides showed that when R=phenyl, the rate of hydrolysis of the thionimide to imide was considerably slower than when R=H (Table 1).[18] Further, N-phenyl substitution renders the simple thionimide more effective as a radiation protective agent. Insertion of alkyl groups on the imide nitrogen renders the thionimide molecule more stable to hydrolysis as reported by Witiak et al.[18]

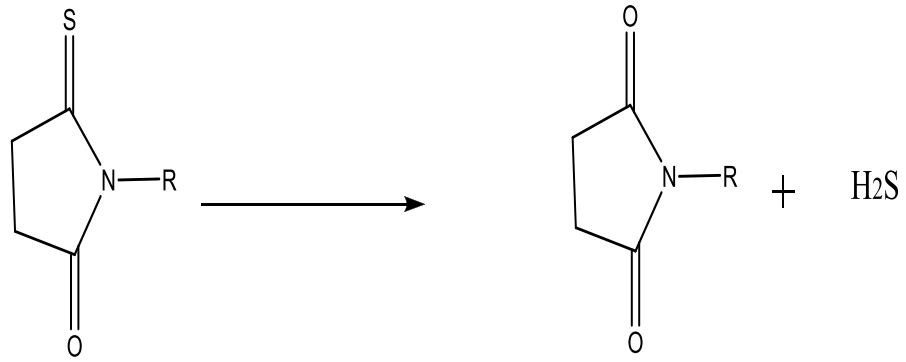


1 2

a, R = H, alkyl, Aryl

b, R_1= O or S

These compounds are less active as radioprotective agents than the corresponding aryl substituted thionimide derivative.[22] In addition, it was reported earlier [23] that the parent system α- aminosuccinimide is not a particularly stable molecule.



3 4

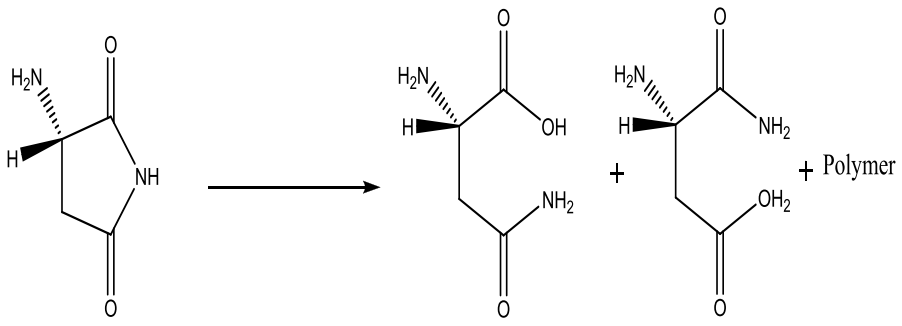
The α-aminosuccinimide was hydrolyzed by heating in aqueous solution for 90 minutes at 80 ^{o}C affording asparagine **5**, isoasparagine **6** and polymeric products. In considering the kinetic results, biological data and the stability of the parent compound **5**, we assumed that α- amino-N-phenylmonothionsuccinimide and α-amino-N-phenyldithionsuccinimide (**1**, **2**) were the best candidates for evaluation of radioprotective activity in this series, for the following;

The following reasons are listed in support of this thought

1- Introduction of an aromatic group on the imide nitrogen should increase the stability of the C=S group [18] as well as the stability of imide ring. Therefore, α-amino-N-phenylmonothionsuccinimide and α-amino-N-phenyldithiosuccinimide may be sufficiently stable in vivo and exert their effects as free radical trapping agents; the highly stabilized free radical **2** may form.

2- N-phenylmonothionsuccinimide is more potent than monothiosuccinimide.[22] We expect that introduction of an amino group in the α-position should further enhance the radioprotective activity of N-phenylmonothionsuccinimide. Aminothiols are more effective than their corresponding thiols.[19]

3- Since α-amino-N-phenylsuccinimide is expected to be more stable than α-aminosuccini-mide. We suspected the phenyl compound would be readily accessible synthetically.

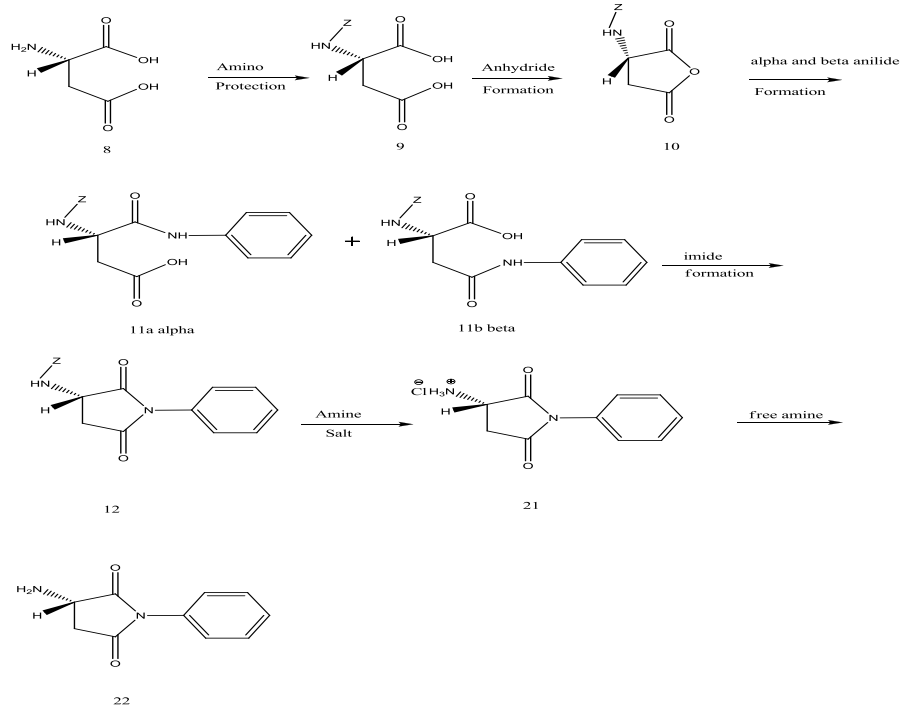


5 6 7

RESULTS AND DISCUSSION

L(S)-aspartic acid **8** served as starting material and was converted in 80% yield to the carbobenzoxy derivative **9** through reaction with benzylchloroformate in the presence of magnesium oxide in water.[24] Derivatization of the amino group was required in order to render the nitrogen atom less basic and prevent its participation in subsequent reactions. The carbobenzoxy (CBZ) group was utilized since it easily removed under conditions employing mineral acid [25, 26] or by catalytic hydrogenation over palladium.[23, 24]

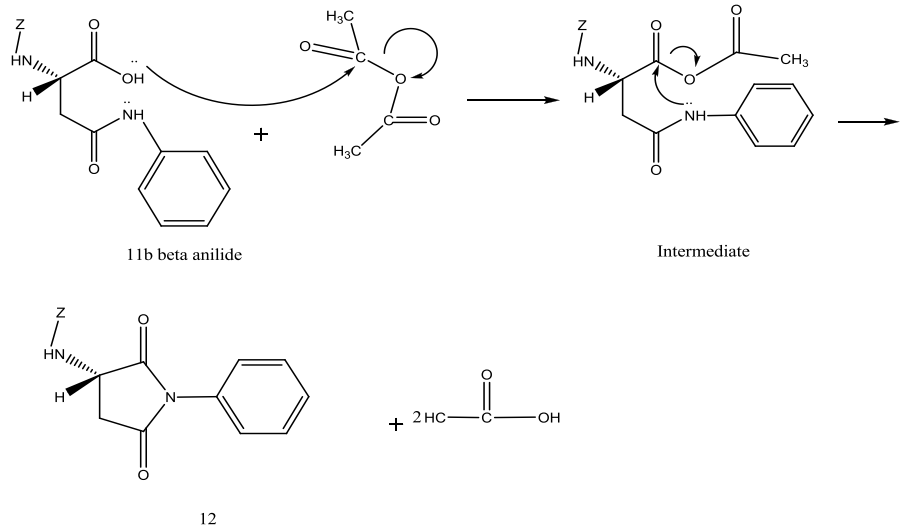
L(S)-aspartic acid **8**, containing α-asymmetric center, was employed since a successful synthesis, not involving racemization would enable preparation of D and L-isomers for biological evaluation. Optical rotatory dispersion (ORD) was employed to follow the reaction sequence. ORD analysis of the carbobenzoxy derivative **9** showed this compound to be formed without racemization (Figure 1). This confirmed the observation of other investigators [27], namely, that the azlacton was not formed during the reaction. Such an intermediate is known to racemize easily under basic conditions.[28, 29]



8 9 10 11a alpha 11b beta 12 21 22

Scheme 1: synthesis of α-amino-N-phenyl succinimide

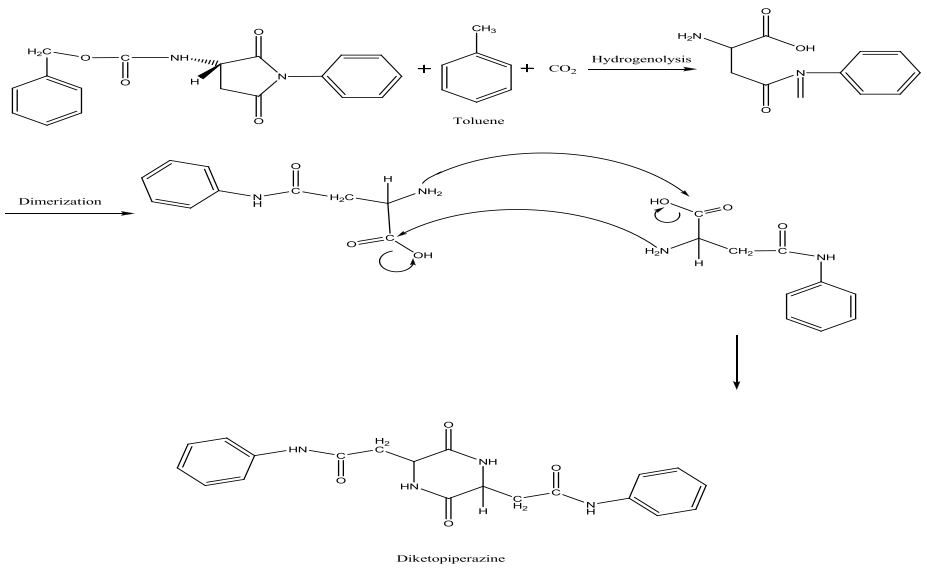
The carbobenzoxy derivative **9** was converted to the corresponding anhydride **10** in 85% yield by heating in acetic acid anhydride. The infrared spectrum was consistent with the assigned structure. Reaction of the anhydride **10** with aniline in absolute ethanol afforded a mixture of α- and β-anilides **11a** and **11b**, respectively.[30, 31] Compounds **11a** and **11b** were heated on steam bath with acetic acid anhydride affording carbobenzoxy-L-amino-N-phrnylsuccinimide **12** in 70% yield. The reaction probably involved the activation of the carboxyl group by mixed anhydride formation **13a** and **13b**; this was likely followed by intramolecular elimination of the acetate ion affording the desired imide **12**. The infrared and NMR spectra were consistent with the assigned structure. The formation of the anilide and the imide is outline in scheme 2. Remove of the protecting group under hydrolysis.



Scheme 2: α & β- anilide conversion to corresponding imide. This one related to β-anilide.

A number of approaches were employed in an attempt to remove the carbobenzoxy protecting group. Catalytic hydrogenation [23, 32] and acid catalyzed hydrolyses [30, 33], under a variety of reaction conditions, were employed without positive results. In general, conditions involving 8-15% HBr in acetic acid for three hours or catalytic hydrogenation over palladium in absolute methanol at room temperature for one to two hours did not afford the free amino imide **12**. Starting material was recovered in quantitative yield. Acid hydrolysis using 30-32% HBr in acetic acid at room temperature for one hour afforded the hydrobromide salt of the anilide **13**; hydrogenation over palladium in absolute methanol for three to four hours at room temperature afforded the α-amino asparatic acid anilide **14.** Hydrogenation over palladium in absolute methanol at room temperature for six hours afforded the diketopiperazine dimer **15.**[23, 34] This dimer was also obtained when the hydrogenation was carried out over palladium in HOAc-in water (1:1) at room temperature for two hours or over palladium in ethyl alcohol-HOAc (1:1) for one-half hour.

In absolute methanol anilide **16** was isolated after four hours of hydrogenolysis. If the reaction was carried out for six hours the diketopiperazine dimer **15** was obtained and the diketopiperazine most likely resulted from dimerization of intermediate **11.**



15

Scheme 3: Removal of the benzyoxcarbonyl of α-amino-N-phenyl succinimide over palladium under acidic condition.

Since the carbobenzoxy group proved difficult to remove without destruction of the imide ring, we resorted to use of the t-butyloxycarbonyl (BOC) function which is more easily hydrolysed under acidic conditions.

Again L(S)-aspartic acid **8** served as starting material. The tertiary butyloxy derivative was obtained in 40% yield by reaction of **8** with T-butylazidoformate.[35] The infrared spectrum was consistent with the assigned structure. The tertiary butyloxycarbonyl-derivative **17** was converted to the corresponding anhydride **18** in 64% yield on a steam bath for one-half hour. Reaction of the anhydride **18** with aniline in absolute ethanol afforded the intermediate α- and β-anilides **19a** and **19b** in a combined yield of 40%. The boc-anilide mixture was heated on a steam bath with acetic acid anhydride for one hour affording the desired BOC-L-amino-N-phrnylsuccinimide **20** in 51% yield. The infrared and NMR spectra were consistent with the assigned structure. Reaction of **20** in trifluroacetic acid [35, 36], followed by treatment with amberlite IRA-400 (ion exchange resin, $RN+(CH_3)_3CL-$) yielded a mixture of the hydrochloride salts of the imide **21** and the anilide **14.** Reaction of boc-L-amino-N-phenylsuccinimide **20** with HCL gas in chloroform-benzene (3:1) for five to ten minutes afforded the hydrochloride salt of L-amino-N-phenylsiccinimide **21** exhibits bands (KBr, cm^{-1}) at 3400 (NH_2, stretch) 3000-2700 (NH_3, broad), 1725 with shoulder at 1780 (C=O, stretch of the imide ring) 1600, 1550, 1500 and 760 (phenyl group). The NMR showed the expected ABX spectrum for the imide ring protons with resonance and coupling constant indicated in table 3. The free amine **22** was liberated from its salt **21** by treatment with 2% sodium bicarbonate solution and extracted into chloroform solution under reduced pressure affording the desired α-amino-N-phenylsuccinimide **22** in 75% yield. The infrared and NMR spectra were consistent with the assigned structure. All attempts to generate α-amino-N-phenylmothionsuccinimide **1** and α-aminodithionsuccinimide **2** with phosphorus pentasulfide under various solvent, temperature and duration of acid lead to degradation of imide ring with no indication that these conditions able to exchange oxygen by sulfur as indicate in some of the experimental part.

EXPERIMENTAL

Melting points are taken on a calibrated Thomas-Hoover melting point apparatus. Infrared spectra are recorded utilizing a Perkin-Elmer 257 spectrophotometer. Optical rotatory dispersion (ORD) are taken utilizing the Durham-Jasco ORD/CD instrument. NMR spectra are recorded on Varian A-60A spectrophotometer. Elemental analysis are performed by Clark Microanalytical Lab, Urbana, Illinois.

N-Carbobenzoxy-L-aspartic acid 9 was prepared by the method of Bergman and Zervas [24] from L-aspartic acid and benzyl chloroformate affording crystals of **9** (80%), m.p. 118-120°C, lit.[24] 116°C.

N-Carbobenzoxy-L-aspartic acid anhydride 10 was prepared from N-Carbobenzoxy-L-aspartic acid **9** by reaction i**n ace**tic acid anhydride affording crystals of **10** (82%), m.p.90-92°C, lit.[24] m.p. 87°C.

N-Carbobenzoxy-L-aspartic acid β-anilide 11 Carbobenzoxy-L-aspartic acid anhydride **10** (11.8g, 4.3 x 10^{-2} mole) was added to a solution of (9.33g, 0.10 mole) aniline in absolute ethanol. The mixture became warm and a precipitate formed after ½ hour. The mixture was acidified with 1N HCL. The precipitate was separated by filtration, dissolved in 5% $NaHCO_3$, and extracted with ether. The aqueous solution was acidified and the precipitate was removed, washed with water, and dried under reduced pressure at 30°C affording 10.2 g (50%) of a mixture of N-Carbobenzoxy-L-aspartic acid and α- and β-anilides. Repeated recrystallization from 95% ethanol afforded N-Carbobenzoxy-L-aspartic acid β-anilide, m.p. 166-168°C. The infrared spectrum showed bands (KBr, cm^{-1}) at 3400 (NH, stretch), 2900 (CH_2, Stretch), 1720, 1710 9C=O, stretch), 1600, 770 (phenyl).

Anal. Calcd. For $C_{18}H_{18}O_5N_2$; Cm 63.11; H, 5.29; N, 8.24. Found: C, 63.13; H, 5.17; N, 8.24.

Carbobenzoxy-L-amino-N-phenylsuccinimide 12. The mixture of N-carbobenzoxy-L-aspartic acid α- ɣ -β-anilides (**11a, b,** 2.8 g, 8.0 $x10^{-2}$mole) and 20 ml of acetic acid anhydride was heated on steam bath for 1 hour. The acetic acid anhydride and acetic acid was removed under reduced pressure and crystallization of the resulting syrup was induced by addition of benzene. Recrystallization from absolute ethanol afforded 1.9 g (70%), crystals: m.p. 160-162°C. The principle infrared bands (KBr, cm^{-1}) were found at 3350 (NH, stretch), 2900 (CH_2, stretch) and 1725 with shoulder at 1760 (C=O, stretch of the imide ring). NMR (d-DMSO, δ), 7.97 (doublet, 1H, NH, ^{J}NH, α-CH=7.00Hz), 7.35 (multiplet, 10 H, aromatic), 4.53 (octet, 1H, α-CH), 2.96 (quartet, 1H, β-CH, ^{J}α-CH, β-CH cis = 11.90 Hz, ^{J}β-CH_2 (gem) = 18.00Hz), 5.10 (singlet, 2H, CH_2).

Anal. Calcd. For $C_{18}H_{16}O_4N_2$: C, 66.65; H, 4.94; N, 8.67. Found: C, 66.43; H, 4.92; N, 8.76.

Attempted synthesis of L-α-amino-N-phenylsuccinimide 22

A stream of hydrogen was passed through a mixture of (1.12 g, 3.5 x 10^{-2} mole) of carbobenzoxy-N-phenylaspartimide, 200 mg palladium black and 50 ml of absolute methanol at room temperature for 1-1/2 hours.[23, 32] The mixture was filtered and the filtrate was concentrated under reduced pressure. Starting material was isolated in quantitative yield. No other product could be detected.

All attempts to remove the benzyloxycarbonyl from the α-amino group in succinimide results in the opening of the imide ring and the degradation of the imide. So we utilize t-butylazidoformate as protecting group.

N-t-Butyloxycarbonyl-L-aspartic acid 17

L-aspartic acid (**8**, 13.31 g, 0.01 mole) was dissolved in 75 ml of 2N NaOH and mixed with solution of (17.7 g, 0.12 mole) of t-butylazidoformate in 25 ml of methanol. Triethylamine (14 ml) in25 of methanol was added drowise and stirred at 40 ^{O}C for 2 hours and at room temperature for 15 hours.[37] The solution was taken to pH 7 with 2N HCl and the methanol was removed under reduced pressure. The acidified aqueous residue was extracted with ether, dried (Na_2SO_4) and concentrated under reduced pressure. The residue, after shaking with petroleum ether, afforded 10.6 g (40%), crystals m.p. 124-126 ^{O}C. infrared(KBr, cm^{-1}) showed bands at 3405 (NH, stretch), 3000-2500 (OH, stretch of the COOH), 1770, 1740 (C=O, stretch of the α-and β-carboxyl).

Anal. Calcd. $C_9H_{15}O_6N$: C, 46.34; H, 6.43; N, 6.00. Found: C, 46.38; H, 6.43; N, 6.11.

N-t-Butyloxycarbonyl aspartic acid anhydride 18

N-BOC-L-aspartic acid (**17,** 2.15 g, 1.0 x 10^{-2} mole) was added to 30 ml of acetic acid anhydride and heated on steam bath for 30 minutes. The acetic acid anhydride and the acetic acid were removed under reduced pressure. The syrup liquid was crystallized upon addition of Skellyslove B affording 1.5 g (64%), crystals m.p. 108-110 ^{O}C. the infrared spectrum (KBr, cm^{-1}) showed bands at 3350 (NH, stretch), 2950 (CH_2, stretch), 1850, 1780 (C=O, stretch of the carbamate).

Anal. Calcd. For $C_9H_{13}O_5N$: C, 50.32; H, 6.51; N, 6.57. Found: C, 48.37; H, 6.40; N, 6.39.

t-Butyloxycarbonyl-L-aminosuccinic acid α- and β-anilides 19a and 19b

N-BOC-L-aspartic acid anhydride (**18**, 2.15g, 1.0 x 10^{-2} mole) was added to a solution of 91.86 g, 2.0 x 10^{-2} mole) of aniline in 20 ml of absolute ethanol. The mixture was stirred at room temperature for 15 hours and acidified with 1N HCl. A syrupy material was deposited which crystallized on standing for 24 hours at room temperature. The crystals were dissolved in 5% $NaHCO_3$ and the excess aniline was removed by extraction with ether. The aqueous layer was acidified with 1n HCl after being stored at 0 ^{O}C for 24 hours. This afforded 1.2 g (40%) of a mixture containing N-BOC-L aspartic acid-α-anilide and N-BOC-L-aspartic acid β-anilide. Recrystallization from 95% ethanol afforded N-BOC-L-aspartic-β-anilide **19,** m.p. 148-150 ^{O}C. The infrared spectrum (KBr, cm^{-1}) of the anilide showed bands at 3350 (NH, stretch), 3000-2500 (OH, stretch of carboxyl broad), 1710, 1695 (C=O, stretch), 1600, 1550, 770 and 700 (phenyl). The hydrochloride salt of the β-anilide was identified by paper chromatography and the ninhydrin reaction.

Anal. Calcd. For $C_{15}H_{20}O_5N_2$: C, 59.01; H, 6.55; N, 9.18. Found: C, 59.00; H, 6.45; N, 8.98.

BOC-L-α-amino-N-phenylsuccinimide 20

The mixture of α-and β-anilides (**19a**, **19b**, 3.05g, 1.0 x 10^{-2} mole) were heated on a steam bath for 1 hour. The acetic acid anhydride and acetic acid were removed under reduced pressure. Crystallization of; 1.5 g (51%), crystals m.p. 166-168^{o}C. the infrared spectrum (KBr, cm^{-1}) showed bands at 3300 (NH, stretch), 2925 (CH_2, stretch), 1740 with a shoulder at 1760 (C=Om stretch of the imide ring), 1600, 1550, 770 and 700 (phenyl). NMR (d-chloroform, δ), 7.40 (multiplet, 5H, aromatic), 1.45 (singlet, 9H, $(CH_3)_3$-C), 5.61 (doublet, 1H, β-CH, ^{J}α-CH,β-CH trans=6.97 Hz), 3.03 (quartet, 1H, β-CH, ^{J}α-CH, β-CH cis=9.03, ^{J}α-CH_2 $^{(gem)}$=18.00 Hz.

Anal. Calcd. For $C_{15}H_{18}O_4N_2$: C, 62.06; H, 6.2; N, 6.9. Found: c, 61.5; H, 6.44; N, 6.65.

L-α-mino-N-phenylsuccinimide hydrochloride 21

BOC-L-α-amino-N-phenylsuccinimide (**20**, 1.4 g, 5.0 x 10^{-3} mole) was added to 2 ml of trifluroacetic acid. The mixture was stirred at room temperature for 15 minutes. Trifluroacetic acid was removed under reduced pressure and 15 ml of water was added to the residue. The aqueous solution was triturated with IRA-400 (ion exchange resin $(CH_3)_3$-$^{+}$NH—Cl) for 15 minutes. The mixture was filtered and the combined filtrates were lyophilized affording 0.5 g (42%), crystals m.p. 237-240^{O}C.

Anal. Calcd. For $C_{10}H_{11}N_2O_2Cl$: C, 52.97; H, 4.80; N, 12.3; Cl, 15.4. Found: C, 49.85; H, 4.8; N, 11.31; Cl, 13.91.This analysis plus spectra evidence and solubility characteristics indicate the prepared is the hydrochloride salt of aspartic acid β-anilide.

Anal. Calcd. For the anilide salt $C_{10}H_{13}O_3N_2Cl$: C, 49.09; H, 5.31; N, 11.41; Cl, 14.53.

L-α-amino-N-phenylsuccinimide hydrochloride 22

BOC-L-amino-N-phenylsuccinimide (**21**, 1.45 g, 5 x 10^{-3} mole) was dissolved in a mixture of chloroform benzene (3:1). Hydrochloric acid (gas) was passed through the solution with continuous stirring. After 10 minutes a white crystalline compound began to precipitate. The mixture was allowed to stand at room temperature, filtered and recrystallized from methanol-ethanol (1:3) affording 6.8 g (75%), crystals m.p. 248-250^{O}C. the infrared spectrum (KBr, cm^{-1}) exhibits bands at 3400 (NH_3, stretch), 300-2700 (NH_3, stretch broad), 17,250 with shoulder at 1780 (C=O, stretch of the imide ring), 1600, 1550, 750 (phenyl). NMR (d-H_2O, δ), 7.50 (multiplet, 5H, phenyl), 4.78 (quartet, 1H, α-CH), 3.35 (quartet, 1H, β-CH_2, ^{J}α-CH, β-CH trans=5.14 Hz), 3.31 9quartet 1H, β-CH, ^{J}α-CH, β-CH cis = 0.36 Hz, ^{J}β-CH_2 $^{(gem)}$= 18.50 Hz).

Anal. Calcd. For $C_{10}H_{11}N_2O_2Cl$: C, 52.97; H, 4.8; N, 12.3; Cl, 15.4. Found: C, 52.45; H, 4.97; N, 12.0; Cl, 15.24.

L-α-amino-N-phenylsuccinimide 22: α-amino-N-phenylsuccinimide hydrochloride (**22**, 1.13 g, 5.0 x 10^{-3} mole) was suspended in 20 ml of chloroform. The mixture was cooled (in ice) and 2% sodium bicarbonate solution was added dropwise until the solution became clear. The chloroform layer was separated and the aqueous layer repeatedly extracted with chloroform. The chloroform fractions were combined, dried (Na_2SO_4), filtered and the chloroform was removed under reduced pressure affording 6.5 g (52.2%) white crystals, m.p. 124-125^{O}C. the infrared spectrum (KBr, cm^{-1}) showed bands at 3350, 3300 (NH_2, stretch), 2900 (CH_2, stretch), 1710 with shoulder at 1775 9C=O, stretch of the imide ring), 1600, 1500, 1494, and 700 (phenyl). NMR (d-chloroform, δ), 7.32 (multiplet, 5H, phenyl), 1.76 (singlet, 2H, NH_2), 3.99 (quartet, 1H, α-CH), 2.85 (quartet, 1H, β-CH, ^{J}α-CH, β-CH ^{trans}=5.76Hz), 2.83 (quartet, 1H, β-CH, ^{J}α-CH, β-CH cis = 8.74 Hz, ^{J}β-CH_2 $^{(gem)}$=18.00 Hz).

Anal. Calcd. For $C_{10}H_{10}N_2O_2$: C, 63.01; H, 5.29; N, 14.71. Found: C, 62.96; H, 5.39; N, 14.62.

Attempted synthesis of α-amino-N-phenylmonothiosuccinimide 1

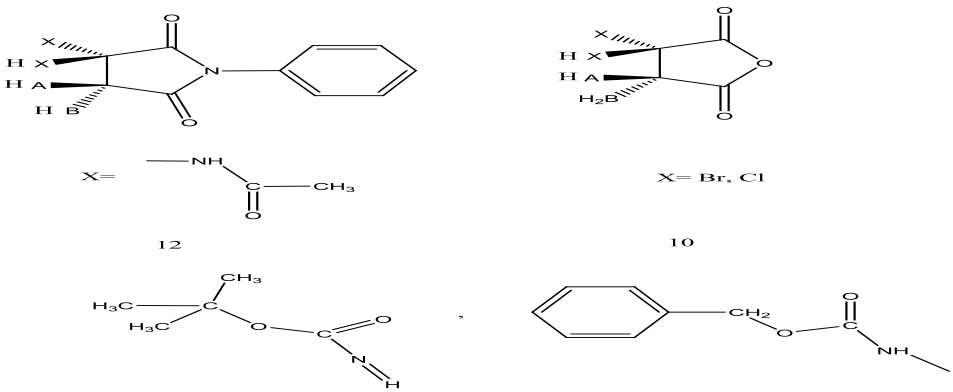
α-amino-N-phenylsuccinimide (**22**, 200 g, 1.0 x 10^{-3} mole) was dissolved in boiling pyridine; and finely powder phosphorus pentasulfide (250 mg, 1.0 x 10^{-3}) was gradually added. The mixture was gently boiled and phosphorus pentasulfide disappeared after 1 hour, leaving an oily mass adhering to the side of the flask. The mixture was filtered while hot. Pyridine was removed under reduced pressure. The residue was dissolved in chloroform (charcoal) and filtered, concentrated under nitrogen pressure and chromatographed on silic acid. Two chloroform fractions in addition to the residue which was eluted with an ethanol-chloroform (1:10) mixture were isolated. The infrared spectrum of the first fraction showed bands (neat, cm^{-1}) at 2910 (CH_2, stretch), 1755 (C=O, stretch), 1500, 1400 (CH_2, bending). The 3300 (NH_2, stretch was lost). The compound does show a positive test for sulfur and negative one for nitrogen, NMR (d-chloroform, δ), no actual structural moiety could be verified. All additional various conditions were attempted to convert imide to thionimide were unsuccessful, and could not results in replacement the imide oxygen by sulfur.

Reaction of α-amino-phenylsuccinimide **21** with H_2S under acidic conditions at 0^{O}C or room temperature [38] afforded starting material in quantitative yield.

First order NMR analysis of allycyclic protons of α-amino-N-phenylsuccinimide 22 and related α-amino derivatives

That part of the NMR spectra attributable to the alicyclic protons of imide compound α-amino-N-phenylsuccinimide **6** may be described in terms of a typical ABX system [39]. The α-protons on the imide – ring exhibits a resonance signal which represents the x-part of the spectrum. The AB part of the spectrum may be assigned to the two geminal protons on the carbon adjacent to the asymmetric center. The signal attributed to the β-proton which is cis to the proton on the asymmetric carbon atom is designed (A) and is located up field to the trans (B) proton resonance signal. A similar ABX spectrum is observed for the methane and methylene proton resonance in the α-acetamido-N-phenylsuccinimide **35c,** α-benzyloxycarbamido-N-phenylsuccinimide **12** and α-t-butyloxycarbamido-N-phenylsuccinimide **17**. However, with these amido-imide derivatives the methane α proton is also coupled with the NH proton of the amido group. Similar observations have been made for cyclic compounds, such as α-substituted succinic anhydride.[40] and bromocyclopropane.[41].

The vicinal coupling constants, summarized in table 3, for the amido derivatives α-benzyloxycarbamide-N-phenylsuccinimide **12** and α-t-butyloxycarbamide-N-phenylsuccinimide **17**, as well as for α-amino-N-phenylsuccinimide hydrochloride **21**, are in agreement with the values assigned by Erickson [40] for vicinal coupling in several mono and disubstituted succinic anhydrides. The vicinal coupling constants for the two monosubstituted succinic anhydrides, where X=OH, Cl, or Br in structure N-Carbobenzoxy-L-aspartic acid anhydride **10**, substantiate cis coupling (8.6-9.0 Hz) to be greater than trans coupling (4.9-6.8 Hz) between the respective (α-methine) and (β-methylene) protons. All observed vicinal coupling constants were in the range 5-9 Hz.



Scheme 4: NMR analysis of allycyclic protons

For the α-amino and α-amido derivatives of N-phenylsuccinimide the greater vicinal coupling constants (which were found in the range 8.74-11.90Hz) were attributed to the cis protons. The smaller coupling constant, which are found in the range 4.74-6.97 Hz for the various succinimide derivatives are assigned to the trans-vicinal protons. These vicinal coupling assignments are in general agreement with those calculated using the Karplus equation. Examination of the deriding molecular models revealed the dihedral angle for the cis protons to be approximately 0 ^{O}C; the dihedral angle for the trans protons was taken to be approximately 145^{O}C. Since these compounds are rigid structures use of these two angles in the Karplus equation predicts cis and trans coupling constants of ~ 9 and 6 Hz, respectively.

Table 3: chemical shift and spin-coupling constants of L-α-amino-N-phenysuccinimide 22 and other related α-benzyloxycarbamide-N-phenylsuccinimide derivatives 12.

Compound	Proton	Chemical shift	Jij	Hz[a]	Solvent
L-α-amino-N-phenylsuccinimide 22	H_X	3.99	$J_{AX}^{(cis)}$	8.74	
	H_B	2.85	$J_{BX}^{(trans)}$	5.76	$CDC1_3$[b]
	H_A	2.83	$J_{AB}^{(gem)}$	18.00	
L-α-amino-N-phenylsuccinimide hydrobromide 21	H_X	4.78	J_{AX}	9.36	
	H_B	3.35	J_{BX}	5.14	D_2O[c]
	H_A	3.31	J_{AB}	18.50	
N-t-Butyloxycarbonyl aspartic acid anhydride 18	H_X	4.32	J_{AX}	9.03	
	H_B	3.01	J_{BX}	6.97	$CDC1_3$[b]
	H_A	3.03	J_{AB}	18.00	
t-Butyloxycarbonyl-L-aminosuccinic acid α- and β-anilides 19a and 19b	H_X	4.63	J_{AX}	11.90	
	H_B	2.96	J_{BX}	5.20	d-DM_{SO}[b]
	H_A	2.91	J_{AB}	18.00	

a Estimated accuracy within ± 0.2 C.P.S. for J_{AX}, J_{BX}, and J_{AB}.
b Chemical shifts are expressed in δ-downshift from internal TMS standard
c Chemical shifts are expressed in δ-downshift from internal 3-9trimethylsily)-propane sulfonic acid sodium salt.

The germinal coupling constants observed for the imide series are quite large (J_{AB} = 18.0 to 18.5 Hz), but similar to those observed for the three monosubstituted succinic anhydride (18.2 to 19.8 Hz). A study of substituted methanes, Barfield and Grant [42] showed that in those compounds with substituents containing π electrons, larger coupling constants are found than predicted using the Gutawsky, Karplus and Grant [43] treatment employing the angular dependence. This increase in the germinal coupling constant, i.e., methyl or methylene, was explained in terms of hyperconjugation. The sign of J_{AB} for these compounds has not been determined, but current opinion for a similar compound, i. e., monosubstituted succinic anhydrides, favor a negative sign.[44-46]

Optical Rotatory Dispersion (ORD)

It has been reported that α-amino acids of the L absolute configuration, when examined in acid medium, show a long wavelength peak at 225 mμ crossover at 210-212 mμ, and a trough at 195-200mμ. This optical rotary dispersion (ODR) corresponds to the n $\longrightarrow$ π* transition of the carboxylchromophore.[47, 48] Since D-amino acids exhibit a similar cotton effect, but of opposite sign, these observation s may readily be correlated with the absolute configuration of amino acids in solution. Benzyloxycarbonyl and t-butyloxycarbonyl groups are now widely used as α-amino protecting groups during peptide and other synthesis. For this reasons, and to determine whether racemization take place during α-amino acid to imide conversion, we studied the optical rotatory dispersion spectra of derivative in the aspartic acid series. A successfully executed synthesis would enable preparation of D and L amino acid imides for biological evaluation.

Prior to the development of instruments capable of measuring the rotatory dispersion well below 240 mμ, various derivatives of amino acids were usually prepared and used for correlation of absolute configuration with the sign of cotton effect. Spectra of dithiocarbamate [49], dimedone [50], pthalolyl [51], N-nitroso [52], and azomethine [53] derivatives of optically pure α-amino acids have been employed for this purpose. A similar derivative of the terminal amino group in a peptide affords rotatory dispersion spectra which reflect the absolute configuration of the N-terminal amino acid. This latter observation holds irrespective of the configuration of the asymmetric carbon atom in the second or subsequently linked amino acids.[54] With our instrument, the hydrochloride salt of L(S)-aspartic acid exhibits a positive plain curve (Figure 4) analogous to the curve reported by Sterm and co-workers.[55] The benzyloxycarbonyl, t-butyloxycarbonyl and the acetyl

derivatives of L(S)-aspartic acid exhibit negative anomalous optical rotatory dispersion curves, with a trough between 240-245 mμ. These ORD spectra correspond to n → π* transitions of the carbonyl group for the benzyloxycarbonyl, t-butyloxycarbonyl and acetamido functions (curves B in Figures 1, 2, and 4, respectively).[56] The β-anilides of these three N-substituted aspartic acids similarly exhibit negative plain curves in the region 270-300 mμ (curve C in the Figure 1, 2, and 5, respectively). This high intensity in the ORD curve in the range of 270-300mμ for all three derivatives apparently reflects the influence of the anilide chromophore on the asymmetric center.[57] The cbz, boc and acetyl derivatives of L(S)-amino-N-pheylsuccinimide exhibit negative cotton curves with troughs near 245mμ. This corresponds to the n → π* transition for the benzyloxycarbonyl, t-butyloxycarbonyl and acetyl functions. The shift to a lower intensity characterizes the anilide to imide conversion, correlates the spectra for these compounds with the L(S)-absolute configuration and suggests the carbonyl chromophore of the protecting group to have a greater influence on the optical rotatory dispersion than does the N-phenylimide function. Removal of the t-butyloxycarbonyl group affords α-amino-N-phenylsuccinimide, the spectrum of which, either as the free base or the hydrochloride salt, exhibits a negative cotton effect with a trough near 320-325mμ. This large shift in the ORD curve to a longer wavelength reflects the removal of the carbonyl chromophore and corresponds to n → π* transition of the N-phenylimido chromophore (Figure 3).[51] Imide hydrolysis, which might take place during protecting group removal, is easily detected since the resulting L(S) aspartic acid β-anilide salt exhibits the expected positive rotatory dispersion, this occurs at the same wavelength of the imide hydrochloride. These studies substantiate the applicability of the synthetic scheme utilizing t-butyloxycarbonyl group for the preparation of D- and L-α-amino-N-substituted succinimides.

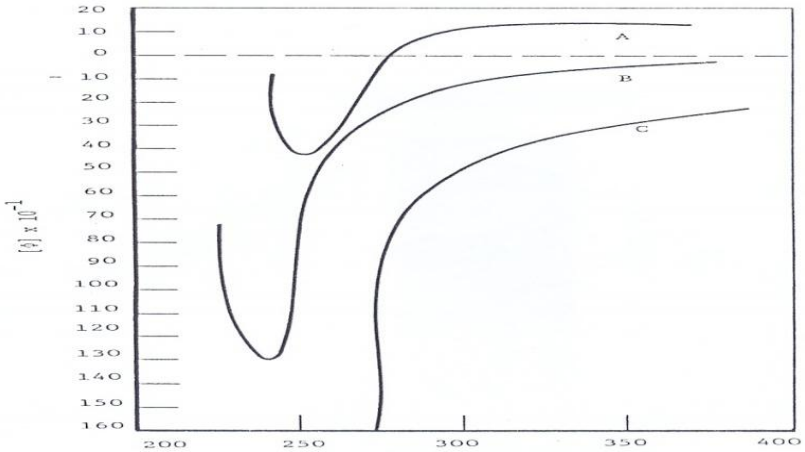


Figure 1. Optical rotatory dispersion spectra in methanol.

A. CbZ-L-α-amino-N-phenysuccinimide (C= 0.012)
B. CbZ-L- α-aminosuccinic acid (C=0.010)
C. CbZ-L- α-aminosuccinic acid β-anilide (C=0.095)

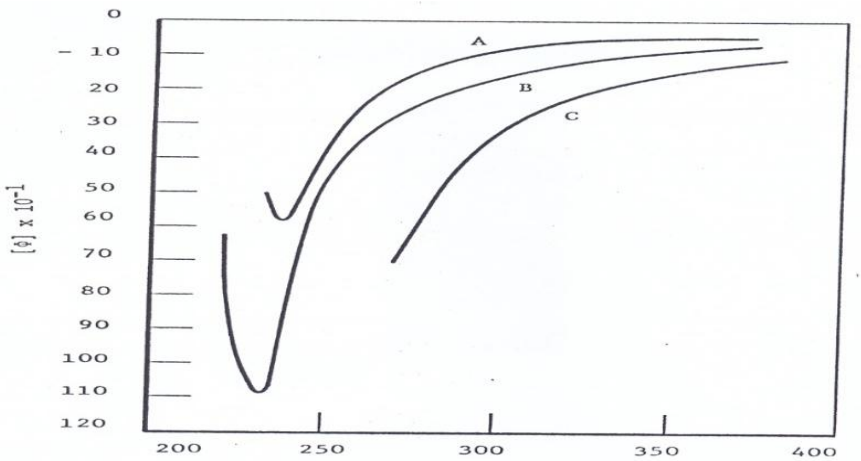


Figure 2. Optical rotatory dispersion spectra in methanol.

A. CbZ-L-α-amino-N-phenysuccinimide (C= 0.010)
B. CbZ-L- α-aminosuccinic acid (C=0.011)
C. CbZ-L- α-aminosuccinic acid β-anilide (C=0.095)

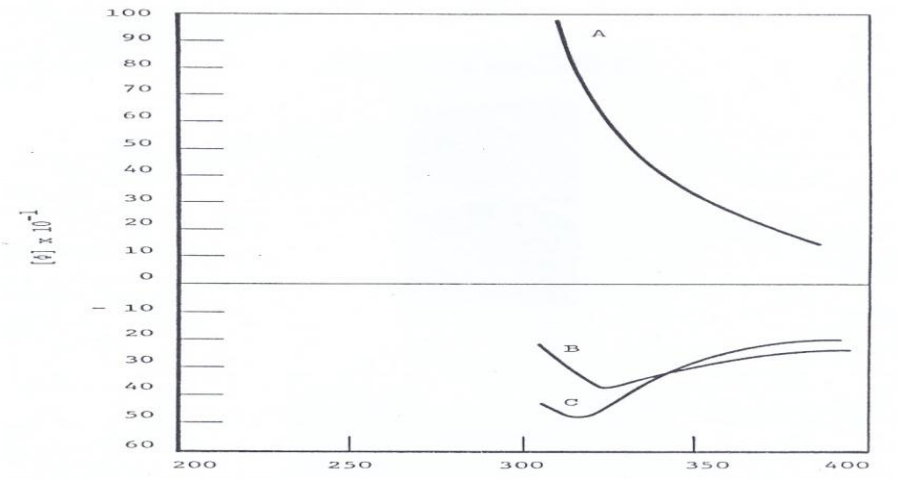


Figure 3. Optical rotatory dispersion spectra in methanol.

A. L- α-aminosuccinic acid β-anilide hydrochloride (C=0.066)
B. L- α-amino-N-phenylsuccinimide (C=0.111)
C. L-α-amino-N-phenysuccinimide hydrochloride (C= 0.042)

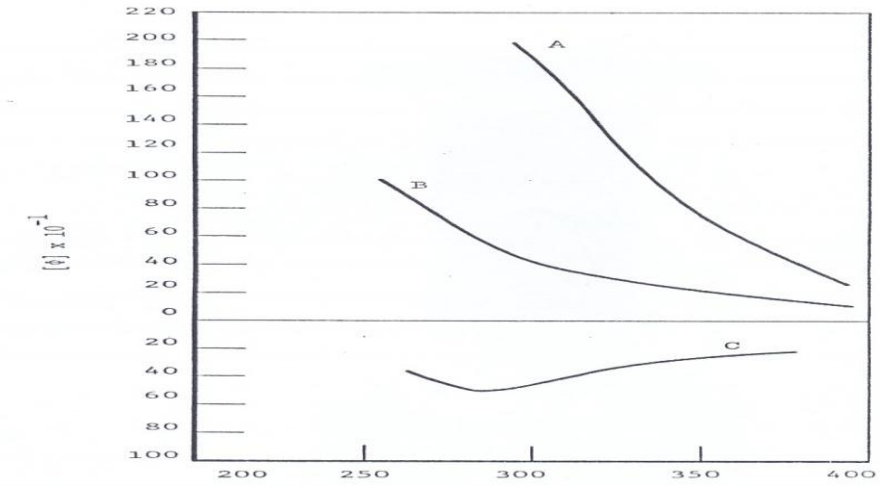


Figure 4. Optical rotatory dispersion spectra in water.

A. L- α-aminosuccinic acid β-anilide hydrochloride (C=0.139)
B. L- α-aminosuccinic acid hydrochloride (C=0.143)
C. L-α-amino-N-phenysuccinimide hydrochloride (C= 0.088)

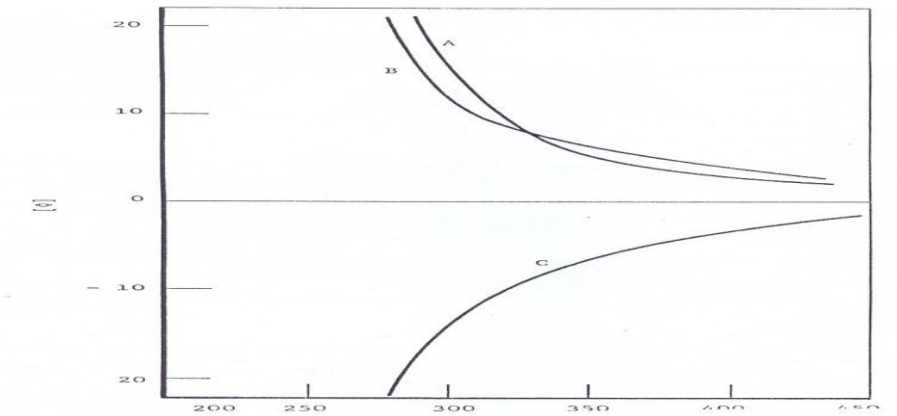


Figure 5. Optical rotatory dispersion spectra in methanol.

A. Methy-L- α-aminosuccinic acid β-anilide hydrochloride (C=0.115)
B. L- α-aminosuccinic acid β-anilide hydrochloride
C. Methy-BOC-L-α-aminoaspartate β-anilide (C= 0.091)

CONFLICT OF INTERESTS
Declared None.

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REFERENCES

1. Phil A, Eldjarn L, Pharmacological aspects of ionizing radiation and chemical protection in mammals.Pharmacol Rev. 1958; 10: 437-74.
2. Lee SJ, Yi CO, Heo RW, Song DH, Cho YJ, Jeong YY, Kang KM, Roh GS, Lee JD. Clarithromycin Attenuates Radiation-Induced Lung Injury in Mice. Plos One 2015; 10 (6): e0131671
3. Cho YJ, Yi CO, Jeon BT, Jeong YY, Kang GM, Lee JE, Roh GS, Lee JD. Curcumin attenuates radiation-induced inflammation and fibrosis in rat lungs. Korean J Physiol Pharmacol, 2013; 17(4): 267-74.
4. Vujaskovic Z, Thrasher BA, Jackson IL, Brizel MB, Brizel DM. Radioprotective effect of amifostine on acute and chronic esophageal injury in rodents. Int J Radiat Oncol Biol Phys. 2007; 6992): 534-40.
5. Cassatt DR, fazenbaker CA, Bachy CM, Kifle G, McCarthy MP. Amifostine (ETHYOL) protects rats from mucositis resulting from fractionated or hyperfractionated radiation exposure. Int J Radiat Oncol Bio Phys. 2005; 61(3): 901-7.
6. Vujaskovic Z, Feng QF, Rabbani ZN, Samulski TV, Anscher MS, Brizel DM. assessment of the protective effect of amifostine on radiation-induced pulmonary toxicity. Exp Lung Res. 2002; 28(7): 577-90.
7. Kurbacher CM, Mallmann PK. Chemoprotection in anticancer therapy: the emerging role of amifostine (WR-2721). Anticancer Res. 1998; 18(3C): 2203-10.
8. Bisht KS, Uma Devi P, Jagetia GC, Kamath G. Drug combination against single drug treatment in radiation protection of the bone marrow CFU. Strahlenther Onkol. 1990; 166(8): 545-8.
9. Maisin JR, Albert C, Henry A. Reduction of short-term radiation lethality by biological response modifiers given alone or in association with other chemical protectors. Radiat Res. 1993; 135(3): 332-7.
10. Maisin JR. Chemical protection against ionizing radiation. Adv Space Res. 1989; 9(10): 205-12.
11. Maisin JR. Protection against ionizing radiation by combinations of radioprotectors. Pharmacol Ther. 1988; 39(1-3): 189-93.
12. Zimmer KG, Ehrenberg L, and Ehrenberg A. Demonstration of long-lived magnetic centers in irradiated biological media & their significance for radiobiology. Stralentherapie, 1957; 103(1): 3-15.
13. Allen AO. The yields of free H and OH in the irradiation of water. Rad Res, 1954; 1 (1): 85-96.
14. Dewhurst HA, Samuel AH, and Magee JL. A theoretical survey of the radiation chemistry of water and aqueous material. Rad Res., 1954; 1: 62.
15. Magee JL. Radiation Chemistry. Ann Rev Nuclear Sci, 1953; 3: 171-92.
16. Bacq ZM, and Alexander P. Fundamental of radiobiology. 2nd ed. Pergamon Press In. New York. 1955; 467-82.
17. P. Alexander, Z. M. Bacq, S. F. Cousens, M. Fox, A. Herve, and J. Lazar. Mode of Action of Some Substances Which Protect against the Lethal Effects of X-Rays. Rad Res. 1955; 2 (4): 392-415.
18. Witiak DT, Chin TF, and Lach JL. Kinetics of hydrolysis of monothionsuccinimides. Org. Chem. 1965; 30 (11): 3721-4. PMID: 5846153[PubMed - indexed for MEDLINE]
19. Doherty DG, Burnett WT, and Shapira R. Chemical Protection Against Ionizing Radiation. II. Mercaptoalkylamines and Related Compounds with Protective Activity. Rad Res. 1957; 7:13.
20. Shapira R, Doherty DG, and Burnett WT. Chemical Protection Against Ionizing Radiation. III. Mercaptoalkylguanidines and Related Isothiuronium Compounds with Protective Activity. Rad. Res. 1957; 7: 22.
21. Lea DE. Actions of Radiation on Living Cells. Cambridge University Press, London, 1955.
22. Williams JR, Little JB. Effects of Ionizing Radiation on Mammalian Cells, In Handbook of Physiology, Reactions to Environmental Agents, John Wiley & Sons, Inc. Published Online: 1 JAN 2011. DOI: 10.1002/cphy.cp090108
23. Sondheimer E, and Holley RW. Imides from Asparagine and Glutamine. J Am Chem Soc. 1954; 76 (9): 2467-70. DOI: 10.1021/ja01638a047
24. Bergmann M, and Zervas L. Über ein allgemeines Verfahren der Peptid-Synthese. Ber. Dtsch. Chem. Ges. 1932; 65: 1192.
25. Bláha K, Frič I and Rudinger J. Amino acids and peptides. XCIV. Optical rotatory properties of diastereomeric cyclohexapeptides containing glycine, leucine, and phenylalanine. Collect. Czech. Chem. Commun. 1969; 34: 3497-509. Doi: 10.1135/cccc19693497
26. Blaha K, and Rudinger J. Amino acids and peptides. XLVII. Rates of fission of some substituted benzyloxycarbonylglycines and two heterocyclic analogues with hydrogen bromide in acetic acid. Collect Czech Chem Commun. 1965; 30 (2): 585-98. doi:10.1135/cccc19650585
27. Bondanszky M, and Ondetti MA. Peptide synthesis. Interscience Publication, New York, 1966 ; 141.
28. Goodman M, and Levine L. Peptide Synthesis *via* Active Esters. IV. Racemization and Ring-Opening Reactions of Opitcally Active Oxazolones. J Am Chem Soc. 1964; 86 (14): 2918-22.
29. Goodman M, and McGahren WJ. Optically Active Peptide Oxazolones. Preliminary Racemization Studies under Peptide-Coupling Conditions. J Am Chem Soc. 1965; 87(13): 3028-9. DOI: 10.1021/ja01091a057.

30. Barker CC. J Chem Soc.The dehydration and racemisation of *N*-acyl-L-aspartic acids by acetic anhydride. J Chem. Soc, 1953; 75 (2): 453-6. DOI: 10.1039/JR9530000453
31. Kovacs J, Kovacs HN, Konyves I, Csaszar J, Vajda T, Mix H. Chemical studies of polyaspartic acid. J Org Chem. 1961; 26: 1084–91.
32. Miller HK, and Waelsch H. The synthesis of glutamine and asparagine peptides and of glutamine. Arch Biochem Biophys. 1952; 35 (1): 176-83.
33. Ben-Ishai D, Berger A. Cleavage of N-Carbobenzoxy Groups by dry Hydrogen Bromide and Hydrogen Chloride J Org Chem, 1952; *17* (12): 1564–70. DOI: 10.1021/jo50012a002
34. Nitecki DE, Halpern B, and Westley JW. Simple route to sterically pure diketopiperazines. J Org Chem, 1968; 33 (2): 864-6. DOI: 10.1021/jo01266a091. PMID: 21894993 [PubMed]
35. Schwyzer R, Iselin B, Kappeler H, Riniker B, Rittel W, und Zuber H. Die Synthese des /i-Melanotropins (/H-MSH) mit der Aminosaurenscquenz des bovinen Hormons. Helv. Chim. Acte, 1963; 46: 1975.
36. McKay FC, and Albertson NF. New Amine-Masking Groups for Peptide Synthesis, J Am Chem Soc, 1957; 79: 4686-90.
37. Mahler HR, and Mehrotra BD. The interaction of nucleic acids with diamines. Biochem Biophys Acta, 1963; 68: 211-33.
38. Duss F, and Lawesson SO, Bowie JH, Cooks RG. Studies on enethiols.2. mass spectra of beta-thioketoesters. arkiv Fur Kemi, 1968; 29 (2): 127.
39. Bovey FA, Mirau P, Gutowsky HS. Nuclear magnetic resonance spectroscopy. Academic press, 1988, pp66.
40. Erickson LE. Proton Nuclear Magnetic Resonance Spectra of Substituted Succinic Anhydrides, Acids, Salts, and Esters. J Am Chem Soc. 1965; 87 (9): 1867-75.
41. Wiberg KB, Nist BJ. The Nuclear Magnetic Resonance Spectra of Cyclopropane Derivatives J Am Chem Soc. 1963; 85 (18): 2788-90. DOI: 10.1021/ja00901a026.
42. Barfield M and Grant DM. The Effect of Hyperconjugation on the Geminal Spin-Spin Coupling Constant. J Am Chem Soc. 1963; 85 (13): 1899-904. DOI: 10.1021/ja00896a001.
43. Gutawsky HS, Karplus M, and Grant DM, Angular Dependence of Electron-Coupled Proton Interactions in CH_2Groups. J Chem Phys.1959; 31: 1278. DOI: org/10.1063/1.73o585.
44. Fraser RR. Relative signs of germinal and vicinal proton-proton coupling constants in α,β-diphenylpropionic acid and its methyl ester. Can. J Chem. 1962; 40: 1483.
45. Anet FAL. The Use of Remote Deuteration for the Determination of Coupling Constants and Conformational Equilibria in Cyclohexane Derivatives. J Am Chem Soc. 1962; 84(6): 1053-54. DOI**:** 10.1021/ja00865a038
46. Lauterbur PC, and Kurland RJ. The signs of CH and HH coupling constants. J Am Chem Soc. 1962; 84 (17): 3405-06.
47. Urry DW, and Eyring H. Optical Rotatory Dispersion Studies of L-Histidine Chelation J. Am. Chem. Soc.1964; 86(21): 4574-80. DOI: 10.1021/ja01075a012
48. Craig JC, and Roy SK. Optical rotatory dispersion and absolute configuration. I. Alpha-amino acids.Tetrahedron 1965; 21 (2): 391-4. PMID: 5888542
49. Sjoberg B, Fredga A, and Djerassi CJ. Optical rotatory dispersion studies .31. anomalous dispersion curves of dithiocarbamates of alpha-amino acids and peptides - stereochemical correlations between alpha-hydroxy and alpha-amino acids Am Chem Soc. 1959; 81; 5002-3.
50. Crabbic P, and Helpern B. Chem. And Ind. 1965, 346.
51. Djerassi C, Lund E, Bunnenberg E and Sheehan JC. Optical Rotatory Dispersion Studies LI. Absolute Configurational Assignments of α-Amino Acids and Peptides through Anomalous Rotatory Dispersion of N-phtha1oyl Derivatives.J. Org. Chem. 1961, 26, 4509.
52. Djerassi C, Lund E, Bunnenberg E, and Sjöberg B. 99. Optical Rotatory Dispersion Studies XLVIII. The Nitroso Chrornophore. J Am Chem Soc. 1961; 83: 2307.
53. Badr Z, Bonnett R, Emerson TR, and Klyne W. J. Optical rotatory dispersion. Part XXI. Azomethines. (I.)*N*-neopentylidene derivatives of α-amino-acid esters. Chem. Soc. 1965. 4503-08. DOI: 10.1039/JR9650004503
54. Djerassi C. Application of anomalous optical rotatory dispersion to organic chemical problems Tetrahedron . 1961; 13 (1-3):13-28. DOI:10.1016/S0040-4020(01)92202-1
55. Sterm J, Krishna Prasad YSR, and Schellman JA. The rotatory dispersion of amino acids. Tetrahedron. 1961; 13 (1-3): 176-84. DOI:10.1016/S0040-4020(01)92211-2.
56. Sakota N, Optical Rotatory Dispersion and Circular Dichroism of Optically Active Acryloyl-a-amino Acids. Nippon Kagada Zasshi, 1968, 89, 425-428.
57. Fric I, Spirko VS, and Blaha K. Amino acids and peptides. LXXXVII. Optical rotatory dispersion of some β-aryl α-amino acids and model compounds. Collect Czech Chem Commun. 1968, 33, 4008-26. DOI:10.1135/cccc19684008