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Ozonolysis of 2-(1,4-Cyclohexadienyl)glycine

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Abstract: Derivatives of 2-(1,4-cyclohexadienyl)glycine were subjected to total ozonolysis. The free esters as well as the N-protected esters tend to cyclize to yield 2-carbethoxy-3-hydroxy pyrrole derivatives. By treatment of the ozonolysis mixture with hydroxylamine or phenylhydrazine heterocyclic derivatives of glycine were obtained. The structure and formation of pyrazolyl and isoxazolyl glycine derivatives is discussed. The structure of N-acetyl-2-(1-phenylpyrazol-3-yl)glycine ethyl ester by X-ray single crystal analysis is reported.

INTRODUCTION

Synthons containing carbonyl groups can be prepared by the ozonolysis of olefins. The ozonolysis of cyclohexadiene[1] and other cyclic dienes were described recently[2]. The combination of Birch reduction and ozonolysis in the transformation of benzene rings in epoxides was described recently by Evans[3]. D-(1,4-Cyclohexadienyl)glycine was prepared by J. Dolfini and cowokers[4] by the Birch reduction of D-phenylglycine. The aim of the present work was to find out whether the ozonolysis of the title compound can lead to either products or synthons containing an amino acid residue. Among other important natural and unnatural compounds that can be achieved by this pathway, are heterocyclic derivatives of glycine. Such derivatives, like ibotenic acid, kainic acid and quisqualic acid were found as agonists of L-glutamic and L-aspartic acids in the mammalian central nervous system[5]. Several other glycine derivatives either posses antibiotic activity like acivicin[6] or they are incorporated in the side chains of penicillins and cephalosporins.

RESULTS AND DISCUSSION

The starting D-cyclohexadienylglycine (2) was prepared from D-phenylglycine (1) by reduction with lithium in liquid ammonia by a slight modification of a procedure described earlier[4]. The free zwitterion was not sufficiently soluble in solvents required for the ozonolysis and its treatment with ozone did not result in identified products. The ethyl ester (3) which was prepared by the esterification of the amino acid 2, was treated with ozone in dichloromethane at -78°C, in the presence of solid sodium bicarbonate as a buffer. The major product which was isolated and identified was 2-carbethoxy-3-hydroxypyrrole (5). The latter was probably formed by the cyclization of the terminal aldehydic group in the intermediate ozonolysis product (4) with the free amino group, as shown in scheme 1. In addition to malonaldehyde which was identified as its dioxime, traces of the oxidized product D-phenylglycine and formic acid were identified.
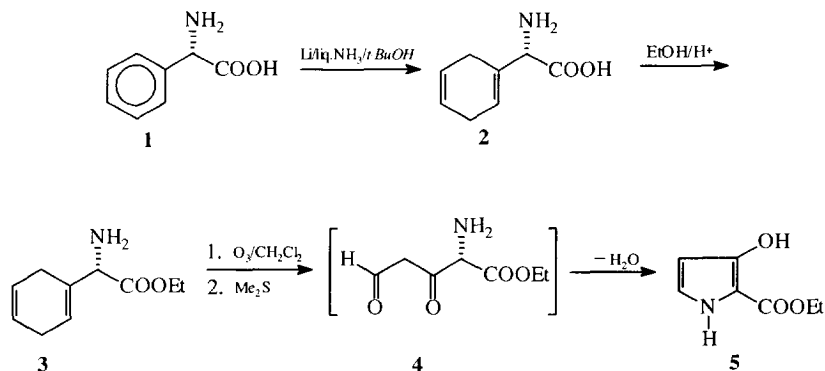
Scheme 1

The ozonolysis of the N-protected substrate, *i.e.* ethyl N-acetyl(1,4-cyclohexadienyl)glycinate (7) was carried out in the same conditions as for its free ester base (3). Column chromatography of the reaction products revealed that the N-acylation did not prevent the cylization to a pyrrole ring. The same pattern of products, including the presence of the pyrrole 5, was observed (scheme 2). However, the major product in this experiment was the equilibrating mixture of 1-acetyl-2-carbethoxy-3-hydroxypyrrole (8a) and 1-acetyl-2H-2-carbethoxy-3-pyrrolinone (8b). In DMSO-d_6 the mixture consisted of a ratio (8a:8b) of 1:4 as shown by ¹H MNR spectra. The tautomeric mixture 8 underwent a facile hydrolysis to yield the pyrrole derivative 5. Acetylation of 8 and 5 afforded 3-acetoxy-1-acetyl-2-carbethoxypyrrole (11) and 3-acetoxy-2-carbethoxypyrrole (10) respectively.
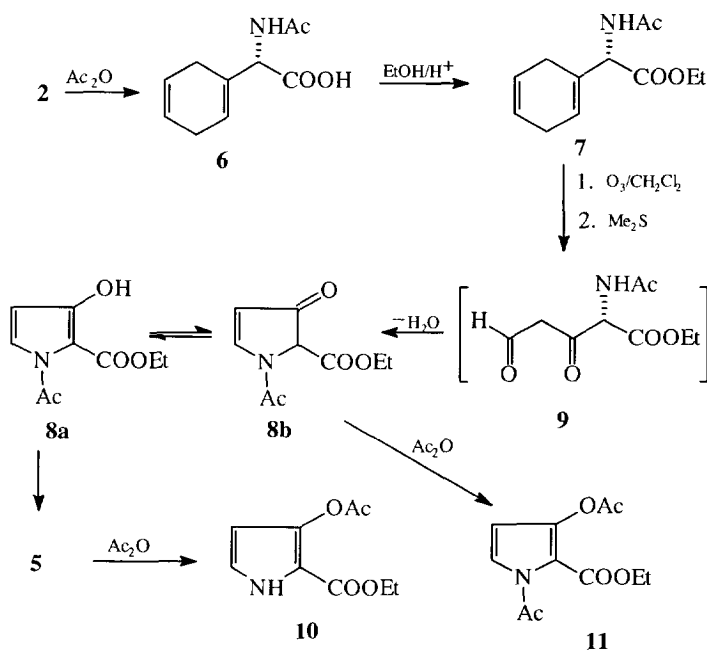
Scheme 2

It was impossible to isolate intermediate **9**, however it was possible to trap it by phenylhydrazine. Upon the reaction with phenylhydrazine in the presence of trifluoroacetic acid it was possible to obtain, in a fair yield the (1-phenylpyrazol-3-yl)glycine derivative (**12**). The formation of this product demonstrates the possibility of obtaining unnatural heterocyclic amino acids in two steps from the available phenylglycine (**1**). However, the optical activity was lost in this procedure. The racemization probably occurs via the enolization of the intermidiate **9** that results in a tautomer (**9a**) which is stabilized by conjugation (Scheme 3). Considering the relative reactivity of the functional groups that take part in the cyclization that leads to the pyrazole ring and that this cyclization should be irreversible, the 5-pyrazolyl isomer (**13**) was expected to be the predominant product. However X-ray crystallography revealed that the pyrazole **12** is the isolated product (**figure 1**). Several explanations are feasible for the mechanism which is responsible for this result. One comprises the deactivation of the NH$_2$ group in phenylhydrazine by protonation, hence the NH group reacts first with the more reactive aldehydic group. A more likely explanation is that the aldehydic group is masked, probably by enolization (e.g. tautomer **9c**), or by a reversible intramolecular cyclization (e.g. to **9b**). This is also demonstrated by the lack of an aldehydic ^{1}H signal in the NMR spectrum of the ozonolysis reaction mixture after the decomposition of the expected ozonides by dimethylsulfide. Such a possible intermediate (**9b**) is shown in scheme 3. Nevertheless a steric effect cannot be excluded as the somewhat bulky phenyl group avoids the more hindered ketonic carbonyl group.
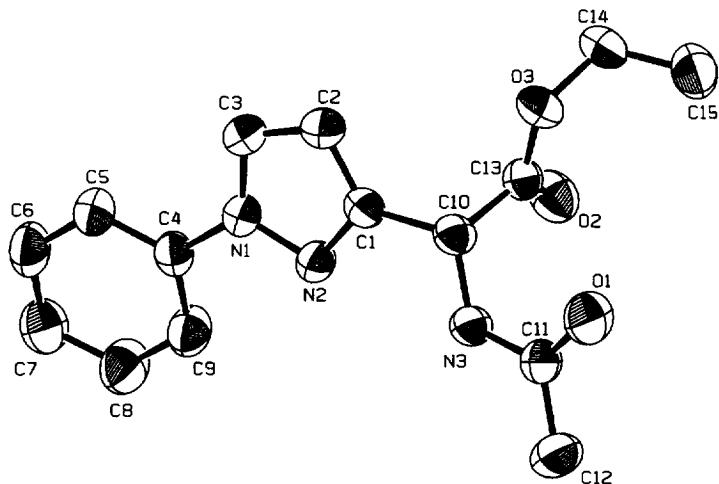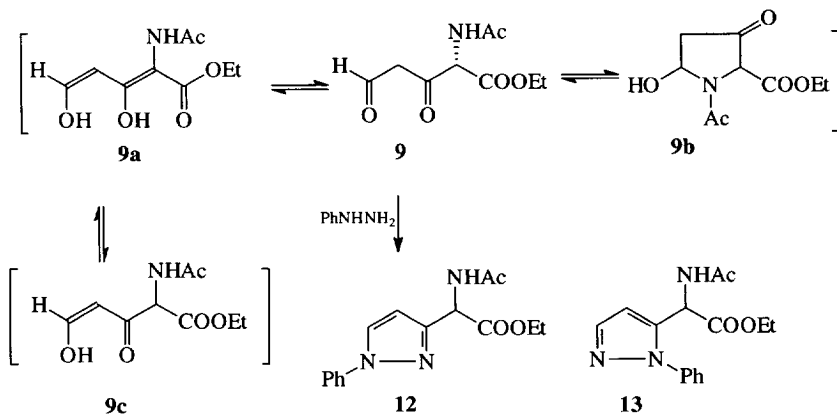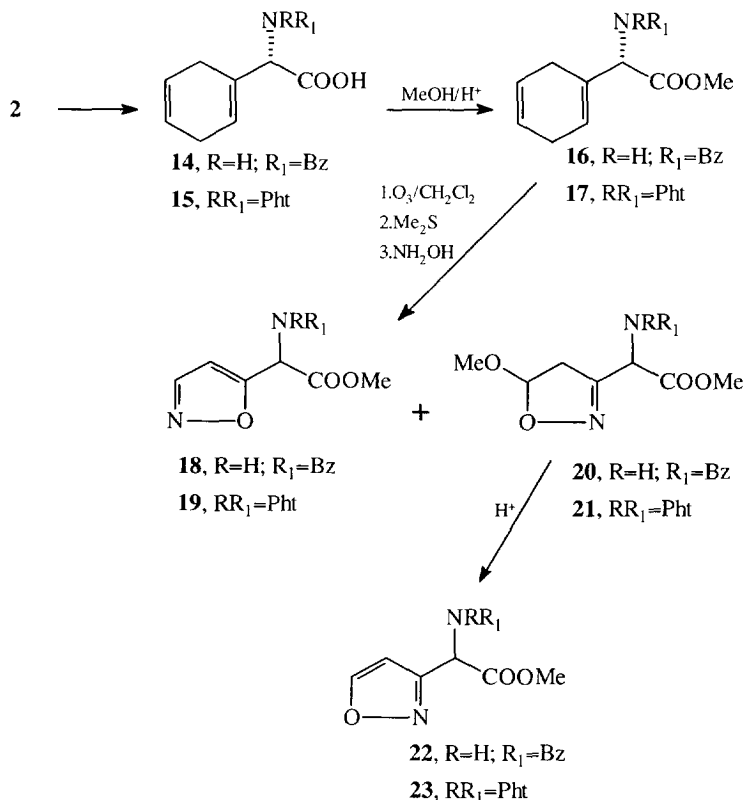
Figure 1: Structure of Ethyl (1-Phenylpyrazol-3-yl)glycinate (12) by X-Ray Crystallography

Scheme 3

In order to prove the generality of this procedure, the introduction of hydroxylamine to produce an isoxazolylamino acid derivative was studied. Thus the N-acetyl ester (7) was subjected to ozonolysis, followed by treatment with hydroxylamine hydrochloride in methanol. The NMR spectrum of the reaction mixture showed the formation of the carbethoxypyrrole derivative (5). However the use of the N-benzoyl ester (16) instead of the N-acetyl ester (7) proved to be more successful. Separation of the reaction products by column chromatography gave only a small amount of the carbethoxypyrrole derivative (5) and the major products were N-benzoyl-2-(isoxazol-5-yl)glycine methyl ester (18) and N-benzoyl(5-methoxy-4H-isoxazolin-3-yl)glycine methyl ester as a mixture of two racemic diastereomers (20a and 20b). The fraction of the ester (18)

was contaminated with traces of its isomer N-benzoylisoxazol-3-yl glycine methyl ester (22). On heating of the isoxazoline derivative (20) in methanolic HCl it underwent elimination of methanol yielding the isoxazolyl derivative (22). The isomeric structure of these amino acids was evidenced by the chemical shifts of the hydrogen nuclei of the isoxazole ring, as there is a considerable difference between the shifts of the hydrogens in position 3 and 5 respectively.

14, R=H; R$_1$=Bz
15, RR$_1$=Pht

16, R=H; R$_1$=Bz
17, RR$_1$=Pht

1.O$_3$/CH$_2$Cl$_2$
2.Me$_2$S
3.NH$_2$OH

18, R=H; R$_1$=Bz
19, RR$_1$=Pht

20, R=H; R$_1$=Bz
21, RR$_1$=Pht

H$^+$

22, R=H; R$_1$=Bz
23, RR$_1$=Pht

Scheme 4

Protection of the amino group with a phthaloyl group was expected to prevent the cyclization to a pyrrole derivative as this cyclization involves the NH group. Preparation of the analogous N-phthaloylcyclohexadienylglycine methyl ester (17) and its ozonoloysis, followed by treatment with hydroxylamine resulted in a mixture of products in which a major part of the phthaloyl group underwent partial hydrolysis. One isomer of the expected N-phthaloylisoxazolylglycine derivative (21) was indeed isolated and identified, however in a low yield. Another fraction consisted of a mixture of two racemic diastereomers (21a and 21b) which was transformed by heating in methanolic HCl to pure isoxazol-3-ylglycine derivative (23). The overall yield of the two isomers was only about 30 %.

CONCLUSION

The ozonolysis products of 2-(1,4-cyclohexadienyl)glycine derivatives tend to undergo cyclization that involves the amino group and the δ-aldehydic group which is formed. However it is possible to trap this products by binucleophiles as phenylhydrazine and hydroxylamine. It is expected that the homologous alanine derivative will show a weaker tendency both to cyclize or racemize. The ozonolysis of the latter is being studied and will be reported shortly.

EXPERIMENTAL

General Methods. Melting points were taken with a Thomas Hoover instrument. NMR was taken with Bruker WP-200 spectrometer. Optical rotation measured with a Perkin-Elmer 141 polarimeter. MS were taken with Varian Mat-311 spectrometer. Chromatographic separation was carried out with silica gel (230-400 mesh) on a 450x10 mm column.

X-Ray Crystal Structure Analysis data were measured on a PW 1100/20 Philips Four-Circle Computer-Controlled Diffractometer, using MoK_α ($\lambda = 0.71069$ Å) radiation with a graphite crystal monochromator in the incident beam was used. The unit cell dimensions were obtained by a least-squares fit of 24 centered reflections in the range $10 \leq \theta \leq 15°$. Intensity data were collected using the $\omega - 2\theta$ technique to a maximum 2θ of $50°$. The scan width, $\Delta\omega$, for each reflection was $1.00 + 0.35 \cdot \tan\theta$ with a scan speed of 3.0 degree/minute. Background measurements were made for a total of 10 seconds at both limits of each scan. Three standard reflections were monitored every 60 minutes. No systematic variations in intensities were found.

Intensities were corrected for Lorentz and polarization effects. All non-hydrogen atoms were found by using the results of the SHELX 86 direct method analysis[7]. After several cycles of refinements[8] the positions of hydrogen atoms were found and added to the refinement process. Refinement proceeded to convergence by minimizing the function $\sum w \, (\, |F_0| \, - \, |F_C| \,)^2$. A final difference Fourier synthesis map showed several peaks less than $0.2 \, e/A^{-3}$ scattered about the unit cell without a significant feature.

The discrepancy indices, $R = \sum w \, (\, |F_0| \, - \, |F_C| \,)/\sum |F_0|$ and $R_W = [\sum w \, (\, |F_0| \, - \, |F_C| \,)^2/\sum w |F_0|^2]^{1/2}$ are presented with other pertinent crystallographic data in Table 1.

Table 1. Crystallographic data[a]:

formula	$C_{15}H_{17}N_3O_3$
space group	I2/a
a, Å	18.319(3)
b, Å	11.971(2)
c, Å	144.117(2)
β, deg	106.52(1)
v, Å^3	2968.0(6)
z	8
ρ_{calcd}, g cm^{-3}	1.29
μ(MoK$_\alpha$), cm^{-1}	0.86
no. of unique reflections	2750
no. of reflections with $I \geq 2\sigma_I$	2040
R	0.043
R$_W$	0.055

[a]Positional parameters, structure factors and U values were submitted to the Editor.

D-2-(1,4-Cyclohexadienyl)glycine (2): A solution of 3.3 g of R-(-)-phenylglycine (1) in 300 mL of NH$_3$ at -78°C was slowly diluted with 80 mL t-butanol. Then lithium (0.5 g) was added in small portions until a permanent blue color persisted. After stirring overnight the remaining solvent was evaporated under reduced pressure. The white residue was dissolved in a small amount of acetone-water, then a solution of chloroform-ethanol 1:1 was added. The mixture was acidified to pH 6 with acetic acid and a white product precipitated. Yield 3.0 g (88 %), m.p. 296-7°C (lit[4]. 297°C), [α]$_D^{25}$= - 89° (c = 1, 2N NaOH, lit[4]. -89.7°).

D-2-(1,4-Cyclohexadienyl)glycine ethyl ester (3): A mixture of D-2-(1,4-cyclohexadienyl)glycine (2, 3.0 g) and 2.3 g of camphorsulfonic acid in 25 mL of ethanol was boiled (3 h) under reflux. Ethanol was evaporated under reduced pressure, the residue was dissolved in water, neutralized by NaHCO$_3$ and extracted with ethyl acetate. Yield: 3 g (oil, 85 %), [α]$_D^{25}$= - 3° (c = 1, chloroform). ^{1}H NMR (CDCl$_3$) δ: 7.34 (bs, 2 H, NH$_2$), 5.70 (bs, 1 H, vinyl), 5.64 (bs, 2 H, vinyl), 4.16 (2 q, J = 7.09 Hz, 2 H), 3.97 (bs, 1 H, CH), 2.58-2.84 (m, 4 H), 1.24 (t, J = 7.09 Hz, 3 H). Anal. Calcd. for C$_{10}$H$_{15}$NO$_2$: C, 66.27; H, 8.34; N, 7.43. Found: C, 66.45; H, 8.03; N, 7.38 %.

2-Carbethoxy-3-hydroxypyrrole (5) from D-1,4-Cyclohexadienylglycine ethyl ester (3): 1,4-Cyclohexadienylglycine ethyl ester (3, 3.0 g) in 10 mL of dichloromethane was added to dichloromethane (25 mL) containing 0.1 g of solid $NaHCO_3$, previously saturated with ozone at -78°C. Ozone was added until blue color persisted. The mixture was washed with nitrogen and 5 mL of dimethyl sulfide was added. The mixture was kept cold for a few hours and then left at room temperature overnight. Sodium bicarbonate was filtered and the dichloromethane was evaporated under redused pressure. The residue was chromatographed with ethyl acetate-petroleum ether 1 : 3. Yield: 0.96 g (37 %). The product was identified by ^1H NMR as 2-carbethoxy-3-hydroxypyrrole (5) which was previously described[9].

N-Acetyl-D-2-(1,4-cyclohexadienyl)glycine (6): D-2-(1,4-Cyclohexadienyl)glycine (2, 2.6 g) was dissolved in 2N NaOH (14 mL). Acetic anhydride (4.2 mL) and 2N NaOH (42 mL) were slowly added with cooling. Then the mixture was stirred for 1 h at room temperature. Upon addition of 6N sulfuric acid (18 mL) with cooling white crystals precipitated. Yield 3.0 g (91 %), m.p. 197-8°C (ethanol-diisopropylether 1:1), $[\alpha]_D^{25}$= -4° (c = 1, ethanol). ^1H NMR (NaOD/D_2O) δ: 5.72 (bs, 3 H, vinyl), 4.55 (s, 1 H, CH), 2.49-2.68 (m, 4 H, CH_2), 1.99 (s, 3 H, acetyl). Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.52; H, 6.83; N, 7.18 %.

N-Acetyl-D-2-(1,4-cyclohexadienyl)glycine ethyl ester (7): A solution of of N-acetyl-1,4-cyclohexadienylglycine (6, 5 g) and 2.2 g of camphorsulfonic acid in 25 mL of ethanol was boiled 3 h under reflux. Ethanol was evaporated, the residue was dissolved in water, neutralized by $NaHCO_3$ and extracted by ethyl acetate. Yield: 3 g (89 %), m.p. 80-81 °C, $[\alpha]_D^{25}$= + 78° (c = 1, dichloromethane). ^1H NMR ($CDCl_3$) δ: 6.24 (d, J = 7.44, 1 H, NH), 5.75 (bs, 1 H, vinyl), 5.65 (bs, 2 H, vinyl), 4.95 (d, J = 7.44 Hz, 1 H, CH), 4.18 (2 q, J = 7.16 Hz, 2 H), 2.65 (m, 4 H), 2.0 (s, 3 H, acetyl), 1.25 (t, J = 7.16 Hz, 3 H). Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.67; N, 6.27. Found: C, 64.84; H, 7.37; N, 6.39 %.

Ozonolysis of N-acetyl-D-2-(1,4-cyclohexadienyl)glycine ethyl ester (7): N-Acetyl-1,4-cyclohexadienylglycine ethyl ester (7, 0.5 g) in 10 mL of dichloromethane was added to dichloromethane (25 mL) containing 0.1 g of solid $NaHCO_3$, previously saturated with ozone at -78°C. Ozone was added until blue color persisted. The mixture was washed with nitrogen and 5 mL of dimethyl sulfide was added. The mixture was kept cold for a few hours and then left at room temperature overnight. Sodium bicarbonate was filtered and the dichloromethane was evaporated under redused pressure. The residue was chromatographed with a solvent gradient (ethyl acetate-petroleum ether from 1 :4 to 1 : 3). The pyrrole (5, 0.09 g) came out first from the column. Then came out the tautomeric mixture of 8a and 8b. Yield: 0.29 g (66 %). ^1H NMR (DMSO-d_6) δ: **8a:** 9.82 (s, 1 H, OH), 7.43 (d, J = 3.70 Hz, 1 H), 5.96 (d, J = 3.70 Hz, 1 H), 4.18 (dq, J_1 = 7.16 Hz, J_2 = 3.55 Hz, 2 H), 2.46 (s, 3 H, acetyl), 1.25 (t, J = 7.16 Hz, 3 H); **8b:** 9.01 (d, J = 4.07 Hz, 1 H), 5.72 (d, J = 4.07 Hz, 1 H), 4.95 (s, 1 H, CH), 4.18(2 q, J = 7.16 Hz, 2 H), 2.31 (s, 3 H, acetyl), 1.25 (t, J = 7.16 Hz, 3 H). MS: 197 (M): 155 (M-42); 109 (100 %). Anal. Calcd. for $C_9H_{11}NO_4$: C, 54.82; H, 5.62; N, 7.05. Found: C, 54.64; H, 5.33; N, 7.05 %.

The mixture of esters (**8**, 0.02g) was refluxed in acetic anhydride (2 mL) for 2 h. Acetic anhydride was removed *in vacuo*. 3-Acethoxy-1-acetyl-2-carbethoxypyrrole (**11**) was obtained upon evaporation. Yield: 0.01 g, (90-95 %). ^{1}H NMR (CDCl$_3$) δ: 7.17 (d J = 3.56 Hz, 1 H,), 6.14 (d, J = 3.56 Hz, 1 H), 4.23 (q, J = 7.13 Hz, 2 H), 2.49 (s, 3 H, N-acetyl), 2.22 (s, 3 H, O-acetyl), 1.25 (J = 7.13 Hz, 3 H).

Upon gentle heating of the tautomeric mixture of esters (**8**) in wet DMSO-d$_6$ or upon leaving on the shelf for a long period in this solvent it hydrolyzed to pyrrole derivative **5**, as identified by NMR.

3-Acetoxy-2-carbethoxypyrrole (10): To a solution of 0.45 g of 2-carbethoxy-3-hydroxypyrrole (**5**) in THF (10 mL) acetic anhydride (0.6 mL) was added and it was heated under reflux with stirring for 15 min. After cooling it was diluted by ice-water and neutralized to pH ~7 by 2N NaOH. The product was extracted with chloroform, dried on MgSO$_4$ and crystallized on evaporation under reduced pressure. Yield 0.46 g (80 %), m.p. 82-3°. ^{1}H NMR (CDCl$_3$) δ: 9.16 (bs, 1 H, NH), 6.80 (t, J = 2.91 Hz, 1 H), 6.08 (t, J = 2.91 Hz, 1 H), 4.26 (q, J = 7.10 Hz, 2 H), 2.27 (s, 3 H), 1.31 (t, J = 7.10 Hz, 3 H). Anal. Calcd. for C$_9$H$_{11}$NO$_4$: C, 54.82; H, 5.62; N, 7.05. Found: C, 55.10; H, 5.90; N, 7.11 %.

N-Acetyl-2-(1-phenylpyrazol-3-yl)glycine ethyl ester (12): 0.5 g of N-acetyl-2,4-cyclohexadienylglycine ethyl ester (**7**) was treated with ozone as above for ester **7**. After treatment with Me$_2$S (5 mL) as above the solvent was evaporated under reduced pressure. Phenylhydrazine (0.6 g) and trifluoroacetic acid (0.2 mL) were added and the mixture was refluxed for 2 min and chromatographed with a solvent gradient (ethyl acetate-petroleum ether from 1 : 4 to 1: 3). The pyrrole (**5**, 0.1 g) came out first from the column. Then came out N-acetyl-2-(1-phenylpyrazol-3-yl)glycine ethyl ester (**12**). Yield: 0.3 g (47 %), white crystals, m.p. 74-5° (diisopropylether). ^{1}H NMR (CDCl$_3$) δ: 7.85 (d, J = 2.48 Hz, 1 H, pyrazole), 7.24 - 7.72 (m, 5 H, Ph), 6.77 (d, J = 7.25 Hz, 1 H, NH), 6.49 (d, J = 2.48 Hz, 1 H, pyrazole), 5.70 (d, J = 7.25 Hz, 1 H, CH), 4.22 (q, J = 6.69 Hz, 2 H), 2.09 (s, 3 H), 1.25 (t, J = 6.69 Hz, 3 H). See also X-ray analysis (Fig. 1). Anal. Calcd. for C$_{15}$H$_{17}$N$_3$O$_3$: C, 62.71; H, 5.96; N, 14.62. Found: C, 62.61; H, 6.12; N, 14.84 %.

N-Benzoyl-D-2-(1,4-cyclohexadienyl)glycine (14): 2.7 g of D-2-(1,4-cyclohexadienyl)glycine (**2**) was dissolved in 25 mL of water. The cooled solution was slowly treated with of benzoyl chloride (1.9 mL) and 2N NaOH (9 mL). Then the mixture was stirred for 1 h at room temperature. 18 mL of 6N sulfuric acid was added with cooling and white crystals precipitated. Yield 3.4 g (84 %), m.p. 173-4°C (ethanol-water), $[\alpha]_D^{25}$= -14° (c = 1, 2N NaOH). ^{1}H NMR (NaOD/D$_2$O) δ: 7.69 (d, J = 7.42 Hz, 2 H, *o*-PhCO), 7.47 (m, 3 H, *m,p*-PhCO), 5.84 (s, 1 H, vinyl), 5.71 (s, 2 H, vinyl), 4.73 (s, 1 H, CH), 2.50-2.70 (m, 4 H, CH$_2$). Anal. Calcd. for C$_{15}$H$_{15}$NO$_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 69.78; H, 5.83; N, 5.38 %.

N-Phthaloyl-D-2-(1,4-cyclohexadienyl)glycine (15): A stirred solution of D-2-(1,4-cyclohexadienyl)glycine (**2**, 2. g) and of sodium carbonate (1.4 g) in 50 ml of water was treated with N-carbethoxyphthalimide (2.7 g). The mixture was stirred until all the reagents dissolved. The solution was filtered and acidified with 6 N HCl. Yield: 2.7 g (72 %). m.p.192-3 °C, $[\alpha]_D^{25}$= - 92° (c = 1, 2N NaOH). ^{1}H NMR (NaOD/D$_2$O) δ: 7.40-7.54 (4 H, phthaloyl), 5.85 (1 H, bs, *vinyl*), 5.76 (2 H, bs, vinyl), 4.75 (1 H, s), 2.64-2.77 (4 H). Anal. Calcd. for C$_{16}$H$_{13}$NO$_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.54; H, 4.41; N, 4.76 %.

N-Benzoyl-D-2-(1,4-cyclohexadienyl)glycine methyl ester (16): A mixture of 3 g of N-benzoyl-1,4-cyclohexadienylglycine and 1.0 g of camphorsulfonic acid in 25 mL of methanol was boiled for 3 h under reflux. The solvent was evaporated under reduced pressure, the residue was dissolved in water, neutralized with NaHCO$_3$ and extracted with ethyl acetate. Yield: 2.5 g (79 %), m.p.93-4 °C, $[\alpha]_D^{25}$= - 55° (c = 1, chloroform). ^{1}H NMR (CDCl$_3$) δ: 7.83 (d, *J* = 6.47 Hz, 2 H, *o*-PhCO), 7.40-7.53 (m, 3 H, *m,p*-PhCO), 6.92 (d, *J* = 7.39 Hz, 1 H, NH), 5.88 (s, 1 H, vinyl), 5.69 (s, 2 H, vinyl), 5.21 (d, *J* = 7.39 Hz, 1 H, CH), 3.40 (s, 3 H, ester), 2.75 (m, 4 H). Anal. Calcd. for C$_{16}$H$_{17}$NO$_3$: C, 70.83; H, 5.16; N, 6.32. Found: C, 71.15; H, 5.21; N, 6.18 %.

N-Phthaloyl-D-2-(1,4-cyclohexadienyl)glycine methyl ester (17): A mixture of 3 g of N-phthaloyl-1,4-cyclohexadienylglycine (**19**) and 1.0 g of camphorsulfonic acid in 25 mL of methanol was boiled for 3 h under reflux. The solvent was evaporated under reduced pressure, the residue was dissolved in water, neutralized with NaHCO$_3$ and extracted with ethyl acetate and the product purified by column chromatography. Yield: 1.4 g (45 %), m.p.83-4 °C, $[\alpha]_D^{25}$= + 48° (c = 1, chloroform). ^{1}H NMR (CDCl$_3$) δ: 7.69-7.86 (m, Hz, 4 H, phthaloyl), 5.85 (bs, 1 H), 5.63 (m, 1 H), 3.75 (s, 3 H, ester), 2.74-2.77 (m, 4 H, 2CH$_2$). Anal. Calcd. for C$_{17}$H$_{15}$NO$_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.59; H, 4.80; N, 4.95 %.

N-Benzoyl-2-(isoxazol-5-yl)glycine methyl ester (18) and N-benzoyl-2-(isoxazol-3-yl)glycine methyl ester (22): N-Benzoyl-D-2-(1,4-cyclohexadienyl)glycine methyl ester (**16**, 0.5 g) was treated with ozone as above for the ester **3**. After treatment with Me$_2$S (5 mL), as above, it was allowed to warm to room temperature overnight. The solution was filtered and the solvent was removed under reduced pressure. The residue was subjected to column chromatography, eluted with a solvent gradient of ethyl acetate - petroleum ether (1 : 10 - 1 : 3). The pyrrole (**5**, 0.07 g) came out first from the column. Two major fractions (**A** and **B**) were isolated. Fraction **A** contained the isoxazole derivative (**18**). Yield: 0.11 g (23 %). ^{1}H NMR (CDCl$_3$) δ: 8.24 (d, *J* = 1.73 Hz, 1 H, isoxazole CH), 7.83 (d, *J* = 6.56 Hz, 2 H, *o*-PhCO), 7.41-7.55 (m, 3 H, *m,p*-PhCO), 7.28 (d, *J* = 7.45 Hz, 1 H, NH), 6.43 (d, *J* = 1.73 Hz, 1 H, isoxazolic CH), 6.17 (d, *J* = 7.58 Hz, 1 H, CH), 3.83 (s, 3 H, ester). Anal. Calcd. for C$_{13}$H$_{12}$N$_2$O$_4$: C, 60.00; H, 4.65; N, 10.76. Found: C, 60.24; H, 4.55; N, 10.27 %.

Fraction **B** consisted of a 3 : 2 mixture of the isoxazole derivative (**18**) and the isoxazoline derivative (**20**), respectively. ^{1}H NMR of **20a** (CDCl$_3$) δ: 7.84 (d, *J* = 6.57 Hz, 2 H, *o*-PhCO), 7.38-7.55 (m, 3 H, *m,p*-PhCO), 5.63 (t, *J* = 6.2 Hz, 1 H, CH), 5.51 (dt, J_1 = 4.16 Hz, J_2 = 0.95 Hz, 1 H, CH), 3.84 (s, 3 H, ester), 3.43 (s, 3 H), 2.9-3.3 (m, 2 H).

The next fraction (Fraction **C**) consisted of a 3 : 1 mixture of stereomers **20a** and **20b**. ¹H NMR of **20b** (CDCl₃) δ: 8.52 (bs, 1 H, NH), 7.84 (d, J = 6.59 Hz, 2 H, o-PhCO), 7.38-7.55 (m, 3 H, m,p-PhCO), 5.64 (t, J = 6.2 Hz, 1 H, CH), 5.50 (dt, J_1 = 4.18 Hz, J_2 = 0.99 Hz, 1 H, CH), 3.79 (s, 3 H, ester), 3.49 (s, 3 H), 2.9-3.3 (m, 2 H). Upon heating of Fractions **B** and **C** in 3N methanolic HCl (10 mL) for 1 h, the mixture of stereomers (**20a** and **20b**) was converted to the isoxazole derivative (**22**). Yield: 0.18 g (35 %, oil). ¹H NMR (CDCl₃) δ: 8.43 (d, J = 1.62, 1 H, isoxazolic CH), 7.83 (d, J = 6.56 Hz, 2 H, o-PhCO), 7.41-7.55 (m, 4 H, m,p-PhCO + NH), 6.55 (d, J = 1.62 Hz, 1 H, isoxazole CH), 6.04 (d, J = 7.49 Hz, 1 H, CH), 3.83 (s, 3 H, ester). Anal. Calcd. for C₁₃H₁₂N₂O₄: C, 59.98; H, 4.65; N, 10.77. Found: C, 59.82; H, 4.46; N, 10.44 %.

Ozonolysis of N-phthaloyl-D-2-(1,4-cyclohexadienyl)glycine methyl ester (17) and reaction with hydroxylamine: N-Phthaloyl-D-2-(1,4-cyclohexadienyl)glycine methyl ester (**17**, 0.5 g) was treated with ozone as above for the ester **3**. After treatment with Me₂S (5 mL), as above, it was allowed to warm to room temperature overnight. The solution was filtered and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (15 mL) and hydroxylamine hydrochloride (0.15 g) was added. The mixture was refluxed for 4 h, cooled, diluted with ice-water and neutralized with sodium bicarbonate. The crude product was extracted with ethyl acetate and chromatographed with a solvent gradient (ethyl acetate-petroleum ether 1 : 10 - 1 : 4). N-phthaloyl-2-(isoxazol-5-yl)glycine methyl ester (**19**) came out first. Yield: 0.05 g (9 %), m.p. 108-9°. ¹H NMR (CDCl₃) δ: 8.27 (d, J = 1.73 Hz, 1 H, isoxazole CH), 7.75-7.95 (m , 4 H, phthaloyl), 6.63 (d, J = 1.73 Hz, 1 H, isoxazole CH), 6.30 (s, 1 H, CH), 3.81 (s, 3 H, ester). Anal. Calcd. for C₁₄H₁₀N₂O₅: C, 58.73; H, 3.52; N, 9.79. Found: C, 58.72; H, 3.80; N, 9.73 %.

Then came out the mixture of **21a** and **21b**. Yield: 0.09 g (17 %), m.p. 156-7°. ¹H NMR (CDCl₃) δ: 7.76-7.93 (m, 4 H, phthaloyl, **a, b**), 6.00 (s, 1 H, α-CH, **a**), 5.97 (s, 1 H, α-CH, **b**), 5.53 (m, 1 H (**a+b**)), 3.83 (s, 3 H, ester, **b**), 3.82 (s, 3 H, ester, **a**), 3.46 (s, 3 H, **b**), 3.41 (s, 3 H, **a**), 3.30 (m, 2 H (**a+b**)). Anal. Calcd. for C₁₅H₁₄N₂O₆: C, 56.60; H, 4.43; N, 8.80. Found: C, 57.05; H, 4.31; N, 8.65 %.

N-Phthaloyl-2-(isoxazol-3-yl)glycine methyl ester (23): Upon heating in 3N methanolic HCl (10 mL) for 1 h, the mixture (**21a** and **21b**) was converted to the isoxazole derivative (**23**). Yield: 0.07 (86 %, oil). ¹H NMR (CDCl₃) δ: 8.43 (d, J = 1.75, 1 H, isoxazolic CH), 7.79-7.91 (m, 4 H, phthaloyl), 6.75 (d, J = 1.75 Hz, 1 H, isoxazole CH), 6.31 (s, 1 H, CH), 3.84 (s, 3 H, ester). Anal. Calcd. for C₁₄H₁₀N₂O₅: C, 58.73; H, 3.52; N, 9.79. Found: C, 58.63; H, 3.71; N, 9.66 %.

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