

# Ozonolysis of (cyclohexa-1,4-dienyl)-L-alanine.<sup>†</sup> An approach to the synthesis of new unnatural amino acids. X-Ray molecular structure of 2-hydroxy-7-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine

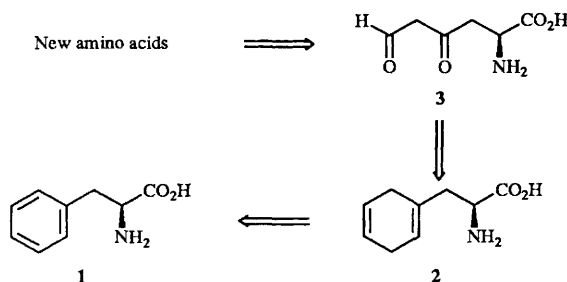
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The aromatic ring in L-phenylalanine was transformed into isoxazolyl, *N*-phenylpyrazolyl, and the bicyclic pyrazolo[1,5-*a*]pyrimidinyl groups, by a combination of Birch reduction and ozonolysis, followed by condensation with the relevant binucleophiles. The ozonolysis was carried out on the *N*-acylated esters of cyclohexa-1,4-dienylalanine. The resulting *N*-acylated esters of heterocyclic alanine derivatives were obtained without isolation of the ozonolysis products. 4-Phenylpyrazolidine-3,5-dione was used as a 'hydrazine donor' in the construction of a pyrazolyl group, as direct condensation with hydrazine was successful.

## Introduction

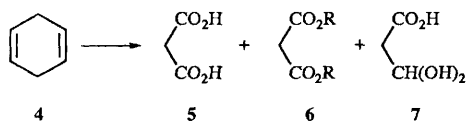
$\alpha$ -Amino acids are enjoying unprecedented renaissance in virtually all disciplines. Many nonproteinogenic amino acids have been found to have biological and pharmacological activities<sup>1</sup> and are also incorporated in semisynthetic penicillins, cephalosporins and in biologically active peptides. The present work presents an approach to the transformation of L-phenylalanine **1** into other optically active amino acids, by a combination of Birch reduction of the aromatic ring, followed by ozonolysis of the resulting cyclohexa-1,4-dienylalanine **2** as shown in the retrosynthetic Scheme 1.



Scheme 1

## Results and discussion

The total ozonolysis of cyclohexa-1,4-diene was described recently<sup>2</sup> by Mittelbach, resulting mostly in the carboxylic acid derivatives **5–7** and negligible amounts of malonaldehyde or its tetraacetal derivative (Scheme 2). However by, using the

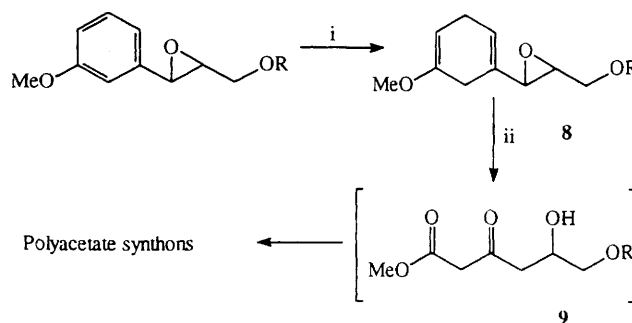


Scheme 2

methodology which is described in the present work, it was shown that it is possible to obtain new amino acids *via* a derivative of intermediate **3** which is obtained in the ozonolysis of the protected L-cyclohexa-1,4-dienylalanine. The yields are fair to good and the optical activity is retained. An analogous procedure which was carried out in this laboratory with

optically active phenylglycine resulted in complete racemization.<sup>3</sup>

A similar strategy was used by Evans<sup>4</sup> in the modification of epoxides, like compounds **8**, in the course of the preparation of synthons which were needed to carry out the syntheses of bryostatin **1** and amphotericin **B** (Scheme 3).

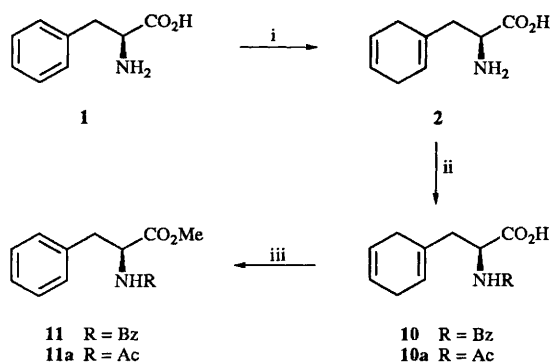


Scheme 3 Reagents: *i*, [H]; *ii*, O<sub>3</sub>

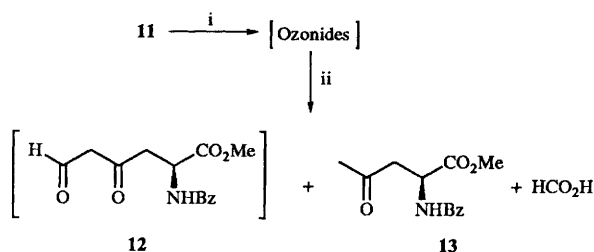
The Birch reduction of L-phenylalanine was carried out with lithium in liquid ammonia and *tert*-butyl alcohol as the proton donor, by a slight modification of a previously described<sup>5</sup> procedure. The reduction was followed by acylation of the amino group, without isolation of the zwitterionic reduction product. The latter was esterified with camphorsulfonic acid (CSA) as a catalyst (Scheme 4). Protection of the amino group in L-phenylalanine before the reduction resulted in partial deacylation, when subjected to the basic conditions of the reduction procedure.

The ozonolysis was performed at  $-78^{\circ}\text{C}$  in dichloromethane, buffered with solid sodium hydrogen carbonate. The solvent was saturated with ozone prior to the introduction of the unsaturated amino acid. After the addition of the unsaturated amino acid, more ozone was introduced until its excess was observed by the appearance of a blue colour. This procedure was utilized to reduce the extent of oxidation of (cyclohexa-1,4-dienyl)-L-alanine derivative **11** to produce methyl *N*-benzoyl-L-phenylalaninate. Starting with solutions saturated with ozone reduced the extent of oxidation to methyl *N*-benzoyl-L-alaninate from about 25% to 2–10%. In addition to the expected pattern to produce dioxo compounds there were some unexpected side products. Among other side products the  $\beta$ -acetylalanine derivative **13** and formic acid were isolated and identified. This derivative is related to a recently described group of 4-oxo- $\alpha$ -amino acids.<sup>6</sup>

<sup>†</sup> Systematic name: 2,5-dihydrophenylalanine.



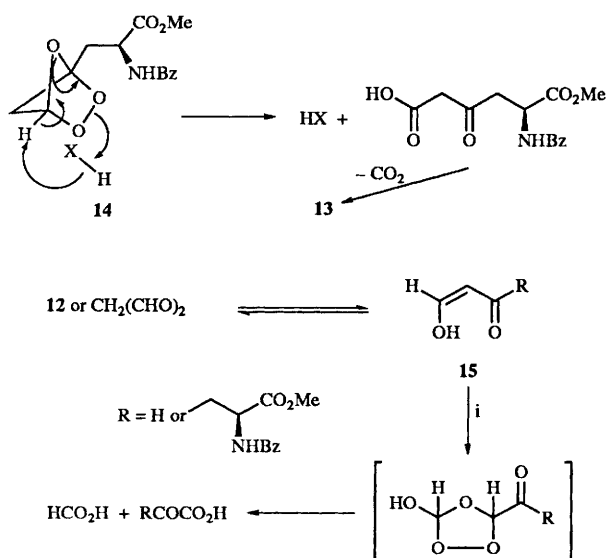
**Scheme 4** Reagents: i, Li, NH<sub>3</sub>, Bu<sup>t</sup>OH; ii, BzCl or Ac<sub>2</sub>O; iii, MeOH, H<sup>+</sup>



Reagents and conditions: i, O<sub>3</sub>, -78 °C; ii, Me<sub>2</sub>S

The proximity of the carbonyl and carbonyl oxide which are formed by the decomposition of the primary ozonide leads to the assumption of the formation of the intermediate **14**. The formation of this bicyclic ozonide is probably preferred to the formation of an intermolecular ozonide. This is also demonstrated by the limited incorporation of methanol when the ozonolysis is carried out in the presence of methanol, neither to form methoxy peroxides nor any acetals. The β-acetylalanine derivative **13** is formed from the ozonide **14**, probably by a mechanism which was proposed by Criegee and Korber<sup>7</sup> which involves acid-base catalysis and is shown in Scheme 5. Formic acid is probably produced by excessive ozonolysis of both intermediate **12** and the malonaldehyde which is obtained from the other three-carbon unit produced in the ozonolysis of the cyclohexadienyl ring, *via* their enolic form **15**.

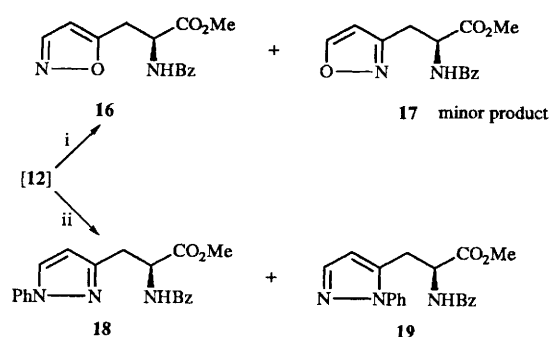
It was impossible either to isolate intermediate **12** or to trap it



**Scheme 5** Reagent: i, O<sub>3</sub>

as the acetal; however, it was possible to induce its reaction with the nucleophiles without isolation of the intermediate products. Schemes 6–8 present the reactions which were carried out to demonstrate the construction of new heterocyclic amino acids. Under the conditions which were chosen for these condensations no racemization was expected. The same pattern of reactions was carried out on the *N'*-(D-α-methylbenzyl)-(cyclohexa-1,4-dienyl)-L-alaninamide derivative **30**, without <sup>1</sup>H NMR-detectable racemization (Scheme 9). The products were isolated by column chromatography, except for the case of the bicyclic pyrazolo[1,5-*a*]pyrimidinylalanine derivative **24**, which was purified by crystallization (Scheme 8).

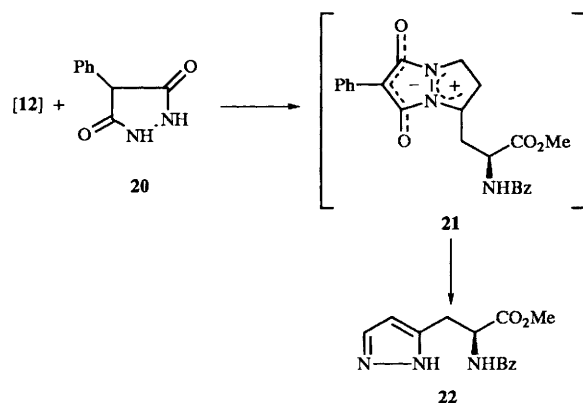
The reaction with hydroxylamine hydrochloride gave mainly the isoxazol-5-ylalanine derivative **16**, whereas phenylhydrazine gave about 1:1 mixture of (1-phenylpyrazol-3-yl)alanine derivative **18** and the (1-phenylpyrazol-5-yl)alanine derivative **19** (Scheme 6). The isomeric structures were determined by <sup>1</sup>H



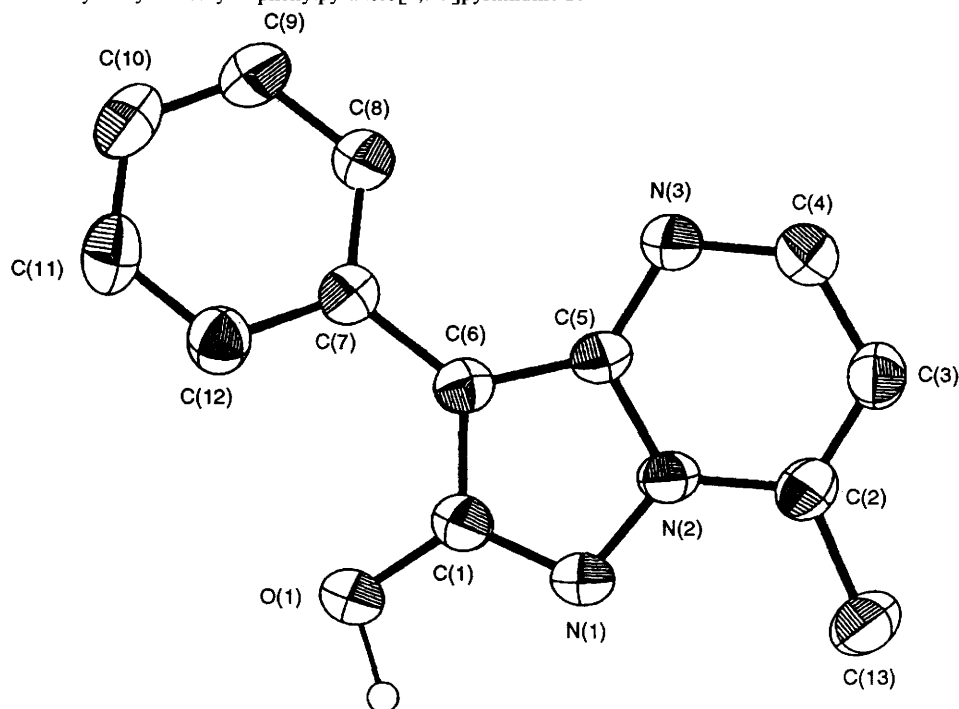
**Scheme 6** Reagents: i, NH<sub>2</sub>OH; ii, PhNHNH<sub>2</sub>

NMR comparison with isoxazole<sup>8</sup> and similar *N*-phenylpyrazole<sup>3,9</sup> derivatives. The yields of these products are moderate; however, the ratio of isoxazoles **16**:**17** could be observed in the NMR spectrum of the reaction mixture, before chromatography was carried out.

Attempts to obtain an unsubstituted pyrazole ring by using hydrazine led to an inseparable mixture of products. In order to synthesize the pyrazole derivative **22**, we took advantage of the convenient reaction of 4-phenylpyrazolidine-3,5-dione **20** with 1,3-dicarbonyl compounds.<sup>10</sup> The resulting paraionic derivatives **21** which are obtained tend to decompose in the presence of secondary amines. Thus, a method was devised for an alternative procedure to the synthesis of the pyrazole derivative **22**. Upon the addition of the phenylpyrazolidine-3,5-dione **20** to the resulting ozonolysis mixture a red colour due to the paraionic intermediate **21** was observed.<sup>10</sup> The decomposition of this intermediate, followed by column chromatography, led to the isolation of the unsubstituted pyrazolylalanine derivative **22** (Scheme 7).

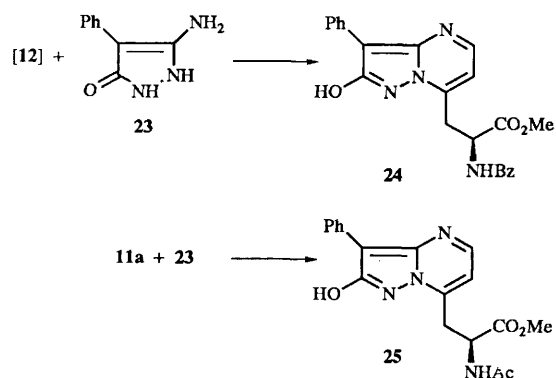


**Scheme 7**

**Table 1** Bond lengths in 2-hydroxy-7-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine **26**

Bond	Distance (Å)	Bond	Distance (Å)
O(1)–C(1)	1.346(2)	C(3)–C(4)	1.394(3)
N(1)–N(2)	1.358(2)	C(5)–C(6)	1.400(3)
N(1)–C(1)	1.328(3)	C(6)–C(7)	1.472(3)
N(2)–C(2)	1.360(3)	C(7)–C(8)	1.389(3)
N(2)–C(5)	1.397(2)	C(7)–C(12)	1.397(3)
N(3)–C(4)	1.329(3)	C(8)–C(9)	1.378(3)
N(3)–C(5)	1.351(2)	C(9)–C(10)	1.377(3)
C(1)–C(6)	1.407(3)	C(10)–C(11)	1.368(3)
C(2)–C(3)	1.355(3)	C(11)–C(12)	1.378(3)
C(2)–C(13)	1.486(3)		

Treatment of the ozonolysis mixture with 5-amino-4-phenyl-1,2-dihydropyrazol-3-one **23** yielded the pyrazolo[1,5-*a*]pyrimidinylalanine derivative **24** (Scheme 8). The regioselectivity of this reaction indicates a considerable difference in the reactivity of the two nucleophilic sites in the aminopyrazole derivative **23**.

**Scheme 8**

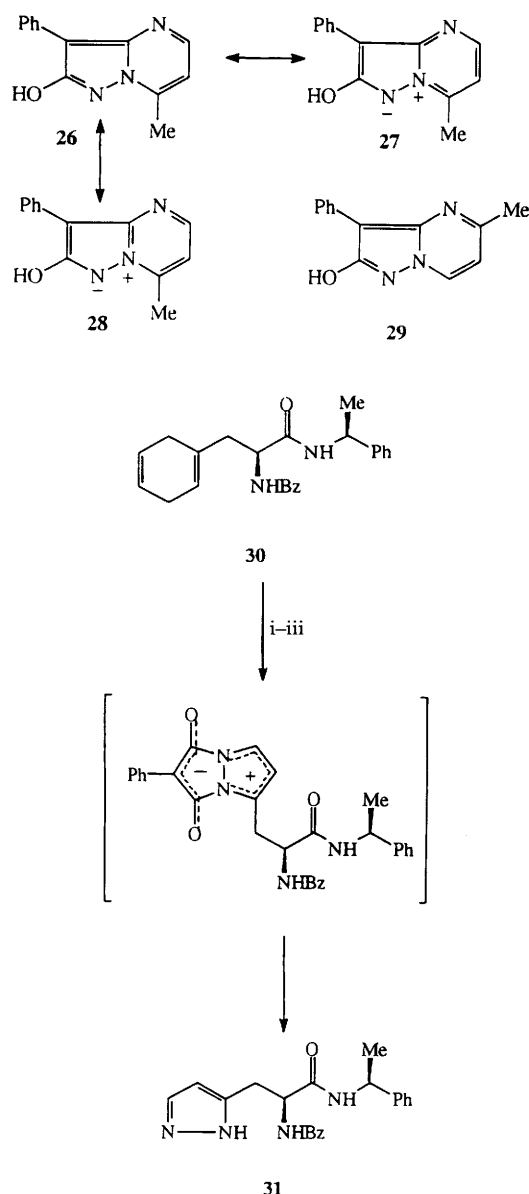
By a similar scheme, the analogous methyl *N*-acetyl 1,4-cyclohexadienyl-L-alaninate **11a** was subjected to ozonolysis, followed by condensation with 3-amino-5-oxo-4-phenyl-2,5-dihydro-1-*H*-pyrazole **23**, leading to methyl *N*-acetyl-(2-

hydroxy-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-yl)-L-alaninate **25**. The structure of both pyrazolo[1,5-*a*]pyrimidine derivatives **24** and **25** was confirmed by analytical and spectral study as well as by comparison with the methyl analogue 2-hydroxy-7-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine **26**, for which a single-crystal X-ray structure determination was carried out. It is unfortunate that compounds **24** and **25** could not be crystallized. This methyl analogue **26** was prepared by the reaction of 3-oxobutyraldehyde diacetal with 3-amino-5-oxo-4-phenyl-2,5-dihydro-1-*H*-pyrazole **23**. Similar systems were described recently by Maquestiau *et al.*<sup>11</sup> Before the final crystallization of this 3-methylpyrazolo[1,5-*a*]pyrimidine derivative **26**, traces of the 5-methyl analogue **29** could be observed in the NMR spectrum of the crude product. Therefore it was possible to assign the relevant signals of the protons and distinguish between the chemical shift of the protons in positions 5 and 7, respectively. This is the first time that such a system has been solved by X-ray diffraction. The bicyclic system is coplanar and, by considering the various bond lengths (Table 1), it may be concluded that there is a substantial delocalization, as expressed by the resonance structures **26–28**.

## Experimental

### General methods

Mps were taken on a Thomas Hoover instrument and are uncorrected. NMR spectra were taken with Bruker WP-200



Scheme 9 Reagents: i,  $O_3$ ; ii,  $Me_2S$ ; iii, **20**

spectrometer.  $J$  Values are given in Hz. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, and  $[\alpha]_D$  values are given in units of  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Chromatographic separation was carried out with silica gel (230–400 mesh) on a  $450 \times 10 \text{ mm}$  column. Light petroleum refers to the fraction with distillation range  $40\text{--}60^\circ\text{C}$ .

X-Ray crystal structure analysis data were measured on a PW 1100/20 Philips four-circle computer-controlled diffractometer, Mo-K $\alpha$  ( $\lambda = 0.71069 \text{ \AA}$ ) radiation with a graphite crystal monochromator in the incident beam. The unit-cell dimensions were obtained by a least-squares fit of 24 centred reflections in the range  $10 \leq \theta \leq 15^\circ$ . Intensity data were collected using the  $\omega$ - $2\theta$  technique to a maximum  $2\theta$  of  $50^\circ$ . The scan width,  $\Delta\omega$ , for each reflection was  $1.00 + 0.35 \tan \theta$  with a scan speed of  $1.5 \text{ degree min}^{-1}$ . Background measurements were made for a total of 20 seconds at both limits of each scan. Three standard reflections were monitored every 60 min. No systematic 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.<sup>12</sup> After several cycles

Table 2 Crystallographic data<sup>a</sup>

Formula	$C_{13}H_{11}N_3O$
Space group	$P2_1/n$
$a(\text{\AA})$	21.741(3)
$b(\text{\AA})$	7.271(1)
$c(\text{\AA})$	6.924(1)
$\beta(^{\circ})$	96.08(2)
$V(\text{\AA}^3)$	1088.4(5)
$Z$	4
$\rho_{\text{calc}}(\text{g cm}^{-3})$	1.38
$\mu(\text{Mo-K}\alpha)(\text{cm}^{-1})$	0.85
No. of unique reflections	2079
No. of reflections with $I \geq 2\sigma_I$	1315
$R$	0.039
$R_w$	0.046

<sup>a</sup> Positional parameters, structure factors and  $U$  values were submitted to the Editor.

of refinement<sup>†</sup> the positions of hydrogen atoms were calculated and added to the refinement process. Refinement proceeded to convergence by minimizing the function  $\sum w(|F_o| - |F_c|)^2$ . A final difference Fourier synthesis map showed several peaks less than  $0.2 \text{ e \AA}^{-3}$  scattered about the unit cell without any significant feature.

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

#### *N*-Benzoyl(cyclohexa-1,4-dienyl)-L-alanine **10**

A solution of L-phenylalanine (6.0 g) in liquid  $NH_3$  ( $300 \text{ cm}^3$ ) at  $-78^\circ\text{C}$  was slowly diluted with Bu'OH ( $100 \text{ cm}^3$ ) during 1 h. Lithium (3.0 g) was added in small portions until the blue colour persisted. Most of the  $NH_3$  evaporated from the stirred mixture overnight at room temperature and the remaining solvent was removed under reduced pressure. The residue was dissolved in a small amount of water and the solution was acidified (to pH 7, using universal indicator) by HCl. Benzoyl chloride ( $4.6 \text{ cm}^3$ ) and 2 mol  $\text{dm}^{-3}$  NaOH ( $20 \text{ cm}^3$ ) were added to the cooled solution portionwise during 45 min. The mixture was then stirred for 1 h at room temperature and acidified by HCl. The precipitated gum was separated by decantation, stirred in a mixture of diethyl ether–light petroleum (2:1) (to remove benzoic acid) and the title compound collected by filtration (6.4 g, 65%), mp  $135\text{--}136^\circ\text{C}$  (Found: C, 70.5; H, 6.3; N, 5.2.  $C_{16}H_{17}NO_3$  requires C, 70.83; H, 6.32; N, 5.16%;  $[\alpha]_D^{25} -4.3$  (c 1, EtOH);  $\delta_H[(CD_3)_2SO]$  12.68 (1 H, s,  $CO_2H$ ), 8.56 (1 H, d,  $J_{NH,\alpha}$  7.2, NH), 7.83 (2 H, d,  $J_{o,m}$  8.1, *o*-PhCO), 7.49 (3 H, m, *m*-Ph + *p*-PhCO), 5.63 (2 H, q,  $J_1$  10.8,  $J_2$  3.2; vinyl), 5.52 (1 H, s, vinyl), 4.56 [1 H, dt,  $J_{\alpha,\beta}$  15.7,  $J(\alpha,NH_2)$  7.2,  $\alpha$ -CH] and 2.46–2.58 (6 H, m,  $\beta$ -CH<sub>2</sub> and cyclohexadienyl CCH<sub>2</sub>).

#### *N*-Acetyl(cyclohexa-1,4-dienyl)-L-alanine **10a**

A solution of L-phenylalanine (6.0 g) in liquid  $NH_3$  ( $300 \text{ cm}^3$ ) at  $-78^\circ\text{C}$  was slowly diluted with Bu'OH ( $100 \text{ cm}^3$ ) during 1 h. Lithium (3.0 g) was added in small portions until the blue colour persisted. Most of the  $NH_3$  evaporated from the stirred mixture overnight at room temperature and the remaining solvent was removed by reduced pressure. The residue was dissolved in a small amount of water and acidified (to pH 7, using universal indicator) by HCl. Acetic anhydride ( $4.2 \text{ cm}^3$ ) and 2 mol  $\text{dm}^{-3}$  NaOH ( $42 \text{ cm}^3$ ) were added to the cooled solution portionwise during 45 min. The mixture was then stirred for 1 h at room temperature. On addition of conc. hydrochloric acid and cooling, lustrous needles of compound **10a** precipitated (6.0 g,

<sup>†</sup> All crystallographic computing was done on a VAX9000 computer at The Hebrew University of Jerusalem, using the TEXAN Structure Analytical Software.



79%; lit.,<sup>5</sup> 58%), mp 179–180 °C (lit.,<sup>5</sup> 163–169 °C) [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 11 (c 1, MeOH) {lit.,<sup>5</sup> [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 10.7 (c 1, MeOH)};  $\delta_{\text{H}}$ (NaOD/D<sub>2</sub>O) 5.79 (2 H, s), 5.59 (1 H, s), 4.30 (1 H, dd,  $J_1$  10.0,  $J_2$  4.5), 2.25–2.66 (6 H, m,  $\beta$ -CH<sub>2</sub> + cyclohexadienyl=CCH<sub>2</sub>) and 2.02 (3 H, s, Ac).

#### Methyl *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alaninate 11

A solution of *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alanine **10** (3.0 g) and CSA (1.3 g) in methanol (50 cm<sup>3</sup>) was heated for 3 h under reflux. Methanol was evaporated off, the residue was dissolved in water, and the solution was neutralized (to pH 7, using universal indicator) by NaHCO<sub>3</sub> and extracted with 3 portions (50 cm<sup>3</sup>) of ethyl acetate. The product crystallized upon concentration under reduced pressure (3.1 g, 100%), mp 76–77 °C (Found: C, 71.7; H, 6.8; N, 4.9. C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub> requires C, 71.56; H, 6.71; N, 4.91%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 32 (c 1, CHCl<sub>3</sub>);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 7.73 (2 H, d,  $J_{\text{o,m}}$  8.2, *o*-PhCO), 7.35–7.47 (3 H, m, *m* + *p*-PhCO), 6.63 (1 H, br s, NH), 5.64 (2 H, s, vinyl), 5.52 (1 H, s, vinyl), 4.83 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.8,  $J_{\alpha,\beta}$  5.7,  $\alpha$ -CH), 3.72 (3 H, s, CO<sub>2</sub>Me) and 2.46–2.64 (6 H, m,  $\beta$ -CH<sub>2</sub> and cyclohexenyl=CCH<sub>2</sub>).

#### Methyl *N*-acetyl(cyclohexa-1,4-dienyl)-L-alaninate 11a

A solution of 6.0 g *N*-acetyl(cyclohexa-1,4-dienyl)-L-alanine (6.0 g) and CSA (1.0 g) in methanol (25 cm<sup>3</sup>) was boiled for 3 h under reflux. The methanol was evaporated off, the residue was dissolved in water, and the solution was neutralized (to pH 7, using universal indicator) with NaHCO<sub>3</sub> and extracted with 3 portions (25 cm<sup>3</sup>) of ethyl acetate. The product crystallized on concentration under reduced pressure (4.95 g, 77%), mp 67–68 °C (Found: C, 64.2; H, 7.7; N, 6.5. C<sub>12</sub>H<sub>17</sub>NO<sub>3</sub> requires C, 64.55; H, 7.67; N, 6.27%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 39 (c 1, CHCl<sub>3</sub>);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 6.13 (1 H, d,  $J_{\text{NH},\alpha}$  7.3, NH), 5.61 (2 H, s), 5.42 (1 H, s), 4.60 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.3,  $J_{\alpha,\beta}$  5.8,  $\alpha$ -CH), 3.66 (3 H, s), 2.22–2.64 (6 H, m,  $\beta$ -CH<sub>2</sub> and cyclohexenyl CCH<sub>2</sub>) and 1.94 (3 H, s, Ac).

#### Methyl *N*-benzoyl(isoxazol-5-yl)-L-alaninate 16

Methyl *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alaninate **11** (0.5 g) in dichloromethane (10 cm<sup>3</sup>) was added to a saturated solution of ozone in dichloromethane (15 cm<sup>3</sup>) at –78 °C, buffered with NaHCO<sub>3</sub> (0.2 g). More ozone was added until the blue colour persisted. The mixture was flushed with nitrogen, dimethyl sulfide (5 cm<sup>3</sup>) was added, and the mixture 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 cm<sup>3</sup>), hydroxylamine hydrochloride (0.2 g) was added, and the mixture was refluxed for 4 h, then cooled, diluted with ice–water and neutralized (to pH 7, using universal indicator) with sodium hydrogen carbonate. The crude product was extracted with ethyl acetate and the extract was chromatographed with a solvent gradient of ethyl acetate–light petroleum (1:3–1:2) to afford title compound (0.25 g, 52%) (Found: C, 61.4; H, 5.1; N, 10.3. C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub> requires C, 61.31; H, 5.14; N, 10.21%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 67 (c 1, dichloromethane);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 8.14 (1 H, d,  $J$  1.5, pyr-CH), 7.78 (2 H, d,  $J_{\text{o,m}}$  7.8, *o*-PhCO), 7.36–7.50 (3 H, m, *m*-*p*-PhCO), 7.20 (1 H, d,  $J_{\text{NH},\alpha}$  7.5, NH), 6.10 (1 H, d,  $J$  1.5, pyr-CH), 5.11 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.5,  $J_{\alpha,\beta}$  5.5,  $\alpha$ -CH), 3.78 (3 H, s) and 3.50 (2 H, dq,  $J_{\text{gem}}$  14.7,  $J_{\beta,\alpha}$  5.5). The spectrum showed the presence of 8% of the regioisomer **17**;  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 8.34 (1 H, d,  $J$  1.4, pyr-CH), 7.78 (2 H, d,  $J_{\text{o,m}}$  7.8, *o*-PhCO), 7.36–7.50 (3 H, m, *m*-*p*-PhCO), 7.20 (1 H, d,  $J_{\text{NH},\alpha}$  7.5, NH), 6.25 (1 H, d,  $J$  1.4, pyr-CH), 3.75 (3 H, s), 5.11 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.5,  $J_{\alpha,\beta}$  5.5,  $\alpha$ -CH), 3.78 (3 H, s) and 3.50 (2 H, dq,  $J_{\text{gem}}$  14.7,  $J_{\beta,\alpha}$  5.5).

#### Methyl *N*-benzoyl-1-phenylpyrazol-3-yl)-L-alaninate 18 and

#### methyl *N*-benzoyl(1-phenylpyrazol-5-yl)-L-alaninate 19

Methyl *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alaninate **11** (1.0 g)

in dichloromethane (10 cm<sup>3</sup>) was added to a saturated solution of ozone in dichloromethane (25 cm<sup>3</sup>) at –78 °C buffered with NaHCO<sub>3</sub> (0.2 g). More ozone was added until the blue colour persisted. The mixture was flushed with nitrogen, dimethyl sulfide (10 cm<sup>3</sup>) was added, and the mixture 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 tetrahydrofuran (40 cm<sup>3</sup>), phenylhydrazine (0.7 cm<sup>3</sup>) was added together with trifluoroacetic acid (0.5 cm<sup>3</sup>), and the mixture was refluxed for 1 h. The solvent was removed by evaporation under reduced pressure and the residue was chromatographed with a solvent gradient: ethyl acetate–light petroleum (1:10–1:2). The isomer eluted first was methyl *N*-benzoyl(1-phenylpyrazol-3-yl)-L-alaninate **18** (0.45 g, 38%), mp <30 °C (Found: C, 68.4; H, 5.2; N, 11.8. C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> requires C, 68.75; H, 5.48; N, 12.03%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 45 (c 1, CHCl<sub>3</sub>);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 7.83 (1 H, d,  $J$  2.3, pyr-CH), 7.63 (2 H, d,  $J_{\text{o,m}}$  8.7, *o*-PhCO), 7.28–7.46 (8 H, m, *m*-, *p*-PhCO + PhN), 6.27 (1 H, d,  $J$  2.3, pyr-CH), 5.10 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.9,  $J_{\alpha,\beta}$  5.3,  $\alpha$ -CH), 3.73 (3 H, s) and 3.34 (2 H, 2 dd,  $J_{\text{gem}}$  15.1,  $J_{\beta,\alpha}$  5.3).

The second isomer, methyl *N*-benzoyl(1-phenylpyrazol-5-yl)-L-alaninate **19** was eluted later (0.37 g, 31%) as crystals, mp 133–134 °C (from ethyl acetate–light petroleum) (Found: C, 68.3; H, 5.4; N, 12.3%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 48 (c 1, CHCl<sub>3</sub>);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 7.64 (2 H, d,  $J_{\text{o,m}}$  8.7, *o*-PhCO), 7.58 (1 H, d,  $J$  1.6, pyr-CH), 7.28–7.51 (8 H, m, PhN + *m*-, *p*-PhCO), 6.60 (1 H, d,  $J_{\text{NH},\alpha}$  7.2, NH), 6.23 (1 H, d,  $J$  1.6, pyr-CH), 4.95 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.2,  $J_{\alpha,\beta}$  5.9,  $\alpha$ -CH), 3.62 (3 H, s) and 3.41 (2 H, 2 dd,  $J_{\text{gem}}$  15.7,  $J_{\beta,\alpha}$  5.9).

#### Methyl *N*-benzoyl[pyrazol-3(5yl)]-L-alaninate 22

Methyl *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alaninate **11** (0.5 g) in dichloromethane (10 cm<sup>3</sup>) was added to a saturated solution of ozone in dichloromethane (15 cm<sup>3</sup>) at –78 °C buffered with NaHCO<sub>3</sub> (0.1 g). More ozone was added until the blue colour persisted. The mixture was flushed with nitrogen, dimethyl sulfide (5 cm<sup>3</sup>) was added, and the mixture was allowed to warm to room temperature overnight. The solution was filtered and the solvent was removed under reduced pressure. 4-Phenylpyrazolidine-3,5-dione **20** (0.45 g) and acetonitrile (10 cm<sup>3</sup>) were added. After being refluxed for 1 min and then left for 2 days at room temperature, the red solution was treated with morpholine (0.4 cm<sup>3</sup>) and left at room temperature overnight. After filtration the solvent was removed by evaporation and the light yellow residue was chromatographed with a solvent gradient of ethyl acetate–light petroleum (1:1–3:0) to give compound (0.19 g, 40%), **22** as an oil (Found: C, 61.4; H, 5.4; N, 15.2. C<sub>14</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub> requires C, 61.53; H, 5.53; N, 15.38%); [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 51 (c 1, EtOH);  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 9.50 (1 H, s, NH), 7.75 (2 H, d,  $J_{\text{o,m}}$  8.5, *o*-PhCO), 7.64 (1 H, d,  $J_{\text{NH},\alpha}$  7.8, NH), 7.44 (1 H, d,  $J$  2.1, pyr-CH), 7.28–7.43 (3 H, m, *m*-*p*-PhCO), 6.09 (1 H, d,  $J$  2.1, pyr-CH), 5.07 (1 H, dt,  $J_{\alpha,\text{NH}}$  7.8,  $J_{\alpha,\beta}$  5.7,  $\alpha$ -CH), 3.68 (3 H, s) and 3.30 (2 H, 2 dd,  $J_{\text{gem}}$  14.5,  $J_{\beta,\alpha}$  5.7).

#### Methyl *N*-benzoyl-2-hydroxy-3-phenylpyrazolo[1,5-*a*]-pyrimidin-7-yl)-L-alaninate 24

Methyl *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alaninate **11** (0.5 g) was treated with ozone and this was followed by reduction with dimethyl sulfide as above. The solvent was removed under reduced pressure and the residue was dissolved in 2 mol dm<sup>–3</sup> HCl/MeOH (15 cm<sup>3</sup>), 3-amino-5-hydroxy-4-phenyl-2,5-dihydro-1*H*-pyrazole **23** (0.7 g) was added, and the mixture was refluxed for 15 min. The crude product which deposited after the addition of ice–water and cooling was separated by decantation. The gummy residue was dissolved in ethyl acetate and dried on MgSO<sub>4</sub>. The product crystallized on evaporation

and was recrystallized from methanol (0.42 g, 57%), yellow crystals, mp 193–194 °C (from chloroform) (Found: C, 63.2; H, 5.0; N, 13.4.  $C_{23}H_{20}N_4O_4 \cdot H_2O$  requires C, 63.59; H, 5.10; N, 12.90%;  $[\alpha]_D^{25} - 132$  (c 1, EtOH);  $\delta_H[(CD_3)_2SO]$  11.84 (1 H, NH/OH), 9.03 (1 H, d,  $J_{NH,\alpha}$  7.8, NH), 8.49 (1 H d,  $J$  4.4, pyr-CH), 8.24 (2 H, d,  $J_{o,m}$  7.5, *o*-PhC), 7.79 (2 H, d,  $J_{o,m}$  8.1, *o*-PhCO), 7.05–7.62 (6 H, m, *m*-, *p*-PhC + *m*-, *p*-PhCO), 6.85 (1 H, d,  $J$  4.4, pyr-CH), 5.24 (1 H, dt,  $J_{\alpha,NH}$  7.8,  $J_{\alpha,\beta}$  4.6,  $\alpha$ -CH), 3.70 (3 H, s) and 3.56 (2 H, dq,  $J_{gem}$  14.5,  $J_{\beta,\alpha}$  4.6).

#### Methyl *N*-acetyl-2-hydroxy-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-yl]-L-alaninate **25**

Methyl *N*-acetyl(cyclohexa-1,4-dienyl)-L-alaninate (0.7 g) was treated with ozone followed by reduction with dimethyl sulfide as above. The solvent was removed under reduced pressure and the residue was dissolved in 2 mol dm<sup>-3</sup> HCl/MeOH (15 cm<sup>3</sup>), 3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazol **23** (0.6 g) was added, and the mixture was refluxed for 15 min. The crude product which deposited after the addition of ice-water and cooling was separated by decantation. The gummy residue dissolved in ethyl acetate and was dried on MgSO<sub>4</sub>. The product crystallized on evaporation. (0.45 g, 42%) as yellow crystals; mp 250–251 °C [decomp, chloroform methanol (10:1)] (Found: C, 60.44; H, 5.3; N, 16.0.  $C_{18}H_{18}N_4O_3 \cdot H_2O$  requires C, 60.66; H, 5.66; N, 15.72%;  $[\alpha]_D^{25} - 103$  (c 1, EtOH);  $\delta_H[(CO_2)_2SO]$  11.76 (1 H, s, NH/OH), 8.50 (1 H, d,  $J_{NH,\alpha}$  8.1, NH), 8.43 (1 H, d,  $J$  4.4, pyr-CH), 8.22 (d,  $J_{o,m}$  8.4, 2 H, *o*-PhC), 7.39 (2 H, dd,  $J_{m,o}$  8.4,  $J_{m,p}$  7.3, *m*-PhC), 7.17 (1 H, t,  $J_{p,m}$  7.3, *p*-PhC), 6.81 (1 H, d,  $J$  4.4, pyr-CH), 4.97 (1 H, dt,  $J_{\alpha,NH}$  8.1,  $J_{\alpha,\beta}$  5.0,  $\alpha$ -CH), 3.64 (3 H, s), 3.46 (2 H, dd,  $J_{gem}$  13.7,  $J_{NH,\alpha}$  5.0) and 1.80 (3 H, s, Ac).

#### 2-Hydroxy-7-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine **26**

1,1-Dimethoxy-3-oxobutane (0.5 g) was dissolved in 2 mol dm<sup>-3</sup> HCl–MeOH (5 cm<sup>3</sup>) 3-amino-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrazole **23** (0.25 g) was added, and the mixture was refluxed for 1 min while the product started to separate. The product was recrystallized from methanol (0.47 g, 72%) as yellow crystals, mp 282–283 °C (from MeOH) (Found: C, 68.7; H, 4.9; N, 18.6.  $C_{13}H_{11}N_3O$  requires C, 69.32; H, 4.92; N, 18.65%;  $\delta_H[(CD_3)_2SO]$  11.76 (1 H, br s, NH/OH), 8.41 (1 H, d,  $J$  4.4, pyr-CH), 8.24 (2 H, d,  $J_{o,m}$  7.3, *o*-PhC), 7.40 (2 H, m, *m*-Ph), 7.15 (1 H, t,  $J_{p,m}$  7.4, *p*-PhCO), 6.90 (1 H, d,  $J$  4.4, pyr-CH) and 2.65 (3 H, s).

#### Methyl L-2-benzoylamino-4-oxopentanoate **13**

The product was isolated upon chromatography in the preparation of compounds **16–19** and **22**. Yields were 5–10% (Found: C, 62.5; H, 6.4; N, 5.7.  $C_{13}H_{15}NO_4$  requires C, 62.64; H, 6.07; N, 5.52%;  $[\alpha]_D^{25} - 24$  (c 1, EtOH);  $\delta_H[(CDCl_3)_2]$  7.81 (2 H, dd,  $J_{o,m}$  8.0,  $J_{o,p}$  1.2, *o*-PhCO), 7.41–7.53 (3 H, m, *m*-, *p*-PhCO), 7.26 (1 H, d,  $J_{NH,\alpha}$  8.1 NH), 4.99 (1 H, dt,  $J_{\alpha,NH}$  8.1,  $J_{\alpha,\beta}$  4.1,  $\alpha$ -CH), 3.77 (3 H, s), 3.23 (2 H, 2 dd,  $J_{gem}$  18.6,  $J_{\beta,\alpha}$  4.1) and 2.19 (3 H, s, Me).

Upon the ozonolysis of the *N*-acetyl derivative **11a** in the preparation of the ester **25** traces of an analogous *N*-acetyl derivative, which was characterized by NMR spectroscopy separated;  $\delta_H[(CDCl_3)_2]$  6.49 (1 H, brd, NH), 4.75 (1 H, dt,  $J_{\alpha,NH}$  8.2,  $J_{\alpha,\beta}$  4.1,  $\alpha$ -CH), 3.71 (3 H, s), 3.11 (2 H, 2 dd,  $J_{gem}$  18.6,  $J_{\beta,\alpha}$  4.1), 2.19 (3 H, s, Me) and 1.99 (3 H, s, *N* Ac).

#### *N*-Benzoyl-*N'*-(*D*- $\alpha$ -methylbenzyl)-L-cyclohexa-1,4-dienyl)-L-alaninamide **30**

To an ice-cooled solution of *N*-benzoyl(cyclohexa-1,4-dienyl)-L-alanine **10** (1.0 g), *D*- $\alpha$ -methylbenzylamine (0.52 g) and 1-hydroxybenzotriazole hydrate (0.6 g) in dichloromethane (50

cm<sup>3</sup>) was added dicyclohexylcarbodiimide (0.8 g). The mixture was then stirred for 1 h at 0 °C and for 6 h at room temperature. The dicyclohexylurea (DCU) which precipitated was filtered off and the filtrate was washed twice with 0.2 mol dm<sup>-3</sup> NaOH (25 cm<sup>3</sup>), twice with water (25 cm<sup>3</sup>), twice with 0.5 mol dm<sup>-3</sup> HCl (40 cm<sup>3</sup>) and again twice with water (40 cm<sup>3</sup>). The organic layer was dried on MgSO<sub>4</sub> and evaporated under reduced pressure. The residue was dissolved in chloroform (25 cm<sup>3</sup>) and an additional amount of DCU was removed by filtration. The product crystallized on concentration under reduced pressure. It contained about 10% of the *D,D*-diastereoisomer. The optically pure product could be obtained by crystallization from ethanol. The *D,D*-isomer was less soluble in cold ethanol (0.88 g, 64%) (Found: C, 77.0; H, 7.0; N, 7.5.  $C_{24}H_{26}N_2O_2$  requires: C, 76.67; H, 6.97; N, 7.42%; *D,L*-isomer:  $\delta_H[(CDCl_3)_2]$  7.69 (2 H, d,  $J_{o,m}$  7.1, *o*-PhCO), 7.15–7.50 (9 H, m, Ph + *m*-, *p*-PhCO + NH), 7.04 (1 H, d,  $J_{NH,\alpha}$  7.7, NH), 5.68 (2 H, s, vinyl), 5.61 (1 H, s, vinyl), 5.05 (1 H, m,  $\alpha$ -CH), 4.84 (1 H, m,  $\alpha$ -CH), 2.71 (4 H, br s, CH<sub>2</sub>), 2.57 (2 H, br d, CH<sub>2</sub>) and 1.44 (3 H, d,  $J$  6.9); *D,D*-isomer:  $\delta_H[(CDCl_3)_2]$  7.71 (2 H, d,  $J_{o,m}$  7.1, *o*-PhCO), 7.15–7.50 (9 H, m, Ph + *m*-, *p*-PhCO + NH), 6.75 (1 H, d,  $J_{NH,\alpha}$  7.4 NH), 5.66 (2 H, s, vinyl), 5.43 (1 H, s, vinyl), 5.05 (1 H, m,  $\alpha$ -CH), 4.84 (1 H, m,  $\alpha$ -CH), 2.70 (4 H, br s, CH<sub>2</sub>), 2.46 (2 H, br d, CH<sub>2</sub>) and 1.47 (3 H, d,  $J$  7.0).

This product was used to assess the absence of racemization on carrying out the general pattern of transformation of cyclohexa-1,4-dienylalanine derivatives into heterocyclic acids, by the following procedure.

A mixture of compound **30** containing 10% *D,D*-diastereoisomer was treated with ozone and 4-phenylpyrazolidine-3,5-dione **20** as in the preparation of pyrazole derivative **22**. The pyrazole derivative **31** which was obtained after aminolysis showed by <sup>1</sup>H NMR spectroscopy the presence of 10% of the *D,D*-diastereoisomer. The *D,L*-isomer:  $\delta_H[(CDCl_3)_2]$  7.70 (2 H, dd,  $J_{o,m}$  8.5,  $J_{o,p}$  1.6, *o*-PhCO), 7.12–7.61 (12 H, m, Ph + *m*-, *p*-PhCO + 2 NH + pyr-CH, NH), 6.19 (1 H, d,  $J$  2.0, pyr-CH), 5.04 (2 H, m, 2  $\times$   $\alpha$ -CH), 3.25 (2 H, br d, CH<sub>2</sub>) and 1.35 (3 H, d,  $J$  7.0); *D,D*-isomer:  $\delta_H[(CDCl_3)_2]$  7.76 (2 H, d,  $J_{o,m}$  8.5, *o*-PhCO), 7.12–7.61 (12 H, m, Ph + *m*-, *p*-PhCO + 2 NH + pyr-CH, NH), 5.93 (1 H, d,  $J$  1.9, pyr-CH), 5.04 (2 H, m, 2  $\times$   $\alpha$ -CH), 3.25 (2 H, br d, CH<sub>2</sub>) and 1.38 (3 H, d,  $J$  7.1).

#### Acknowledgements

We thank Dr Shmuel Cohen for his dedication in carrying out the X-ray diffraction analysis.

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Paper 5/01652E

Received 15th March 1995

Accepted 15th May 1995