126. Determination of the Chemical Structure of Novel Colored 1*H*-Pyrrol-3(2*H*)-one Derivatives Formed by *Maillard*-Type Reactions

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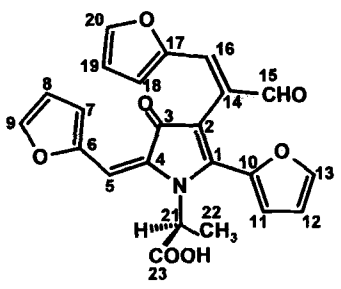
The determination of the chemical structure of a previously unknown *Maillard* reaction product with an amino acid incorporated in a four-ring structure is reported. The red compounds **1a** and **1b**, isolated from a thermally treated aqueous solution of furan-2-carbaldehyde and L-alanine, were identified as (*S*)-4{(*E*)-1-formyl-2-(2-furyl)ethenyl}-5-(2-furyl)-2-{(*E*)-(2-furyl)methylidene}-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid and its 2-{(*Z*)-(2-furyl)methylidene}isomer, respectively, by several 1D- and 2D-NMR techniques, MS, UV, and IR spectroscopy as well as by synthetic experiments. 2D-NOESY and 2D-ROESY experiments performed for conformation analysis indicated the existence of two atropisomers.

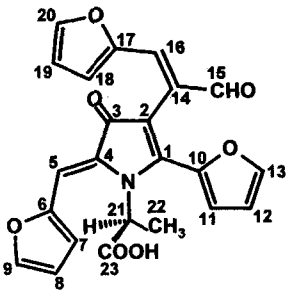
1. Introduction. – Besides the development of typical odors [1], color formation is one of the most important attributes of the *Maillard* reaction between reducing carbohydrates and amino acids occurring, *e.g.*, during thermal processing of foods, such as roasting of meat, baking of bread, or roasting of coffee. However, compared with odorants, surprisingly little is known about the chemical nature of the compounds responsible for the typical brown color. Because of the complexity of the *Maillard* reaction and variety of products formed, it is a helpful approach to study suitable model systems. Such model reactions provide useful informations regarding to the nature of the chromophoric compounds involved in color formation and will help to understand the type of reactions leading to color development. In the last 20 years, only a few investigations [2][3] have been performed to clarify the mechanisms of the so-called nonenzymatic browning; however, most model reactions have been carried out in organic solvents rather than in aqueous solution and, also, used only amines instead of amino acids. Very recently, *Arnoldi et al.* [4] were successful in isolating a yellow colorant from a heated aqueous solution of xylose and lysine. The authors proposed a three-ring carbocyclic structure for the colorant, but although a 1:1 mixture of lysine and xylose was used in the experiments, a lysine moiety was lacking in the colorant. The authors could, however, not suggest from which carbohydrate-derived intermediates the colorant was formed.

To gain more insight into precursors of colored *Maillard* products, it might, therefore, be more promising to identify the chromophoric structures formed by reacting certain intermediates known as carbohydrate degradation products. For example, furan-2-carboxaldehyde is known as one of the main reaction products formed from pentoses during thermal treatment [5]. It was reported that this aldehyde reacts easily with carbohydrate degradation products containing an activated methylene group such as 4-hydroxy-5-methylfuran-3(2*H*)-one giving rise to yellow reaction products [6]. In a very recent

investigation, we showed [7] that the red compound **1a** and, to a smaller content, also the isomer **1b** were formed from a heated aqueous solution of furan-2-carboxaldehyde and L-alanine.



1a[1)]



1b[1)]

As to our knowledge, these compounds have not been described previously in the literature. The details of the determination of the chemical structure of **1a** and **1b** and studies on their conformation are reported in the present investigation.

2. Results and Discussion. – 2.1. *Spectroscopical Characterization.* The determination of the chemical structure was performed by several 1D- and 2D-NMR techniques, and in addition, by MS, UV, and IR spectroscopy. The data were consistent with the structures **1a** and **1b**.

In the following, the structure determination of the predominating isomer **1a** is reported in more detail. The ^{1}H-NMR spectrum of **1a** measured in (D_6)DMSO showed 28 resonance signals in two sets of 14 signals each. The observed 3:2 splitting of the spectrum of **1a** was due to the existence of two conformers $\mathbf{1a}_1$ and $\mathbf{1a}_2$ (for details, see *Chapt. 2.4*). Further NMR data, which are well in line with the assignment of structure **1a**, are given in *Table 1*.

The chemical shift of the 2 *s* at 6.90 and 7.55 ppm for the major conformer $\mathbf{1a}_1$ or at 7.10 and 7.49 ppm for the minor conformer $\mathbf{1a}_2$ were in the expected range for olefinic H-atoms, whereas the *s* at 9.48 ppm was assigned to the H-atom of an aldehyde function. Double-quantum-filtered COSY as well as TOCSY experiments revealed several strongly coupled ^{1}H-spin systems. H,H-Correlations between H–C(21) and Me(22)[1)] at 1.60 and 4.64 ppm ($\mathbf{1a}_1$) or 1.43 and 4.75 ppm ($\mathbf{1a}_2$), respectively, with J = 7.3 Hz confirmed the presence of an alanine moiety in the structure **1a**. The homonuclear correlations with J = 3.5 and 1.4 Hz observed for the ^{1}H-spin systems H–C(7)/H–C(8)/H–C(9), H–C(11)/H–C(12)/H–C(13), and H–C(18)/H–C(19)/H–C(20) corresponded to the pattern expected for furan rings substituted at the 2-position [8]. Three furan rings are, therefore, proposed to be incorporated in structure **1a**. The signal of furan proton H–C(7) of $\mathbf{1a}_1$ at 8.21 ppm was, however, *downfield* shifted by *ca.* 1.3 ppm relative to those of the two corresponding furan protons H–C(11) and H–C(18) which resonate in the expected range at 6.83 and 6.87 ppm, respectively [8]. Since C(7) exhibited no abnormal deshielding in the ^{13}C-NMR spectrum, this unusually large downfield shift of H–C(7) suggests that this proton is in a more deshielding environment than H–C(11) or H–C(18).

The ^{13}C-NMR spectrum of **1a** (*Table 2*) showed 46 signals splitted into two sets of 23 signals (conformers $\mathbf{1a}_1$ and $\mathbf{1a}_2$).

[1)] Arbitrary numbering, as depicted in the *Formulae*; for systematic names, see *Exper. Part.*

Table 1. *Assignment of ^{1}H-NMR Signals* (600 MHz, (D_6)DMSO) *of* **1a**[a])

	δ [ppm][b])		Multiplicity[c])	J [Hz][c])	Connectivity[d]) with
	1a$_1$	**1a$_2$**			
Me(22)	1.60	1.43	*d*	7.3	H–C(21)
H–C(21)	4.64	4.75	*q*	7.3	Me(22)
H–C(19)	6.55	6.58	*dd*	3.5, 1.4	H–C(18), H–C(20)
H–C(8)	6.61–6.63	6.61–6.63	*dd*	3.5, 1.4	H–C(7), H–C(9)
H–C(12)	6.61–6.63	6.61–6.63	*dd*	3.5, 1.4	H–C(11), H–C(13)
H–C(11)	6.83	7.02	*d*	3.5	H–C(12)
H–C(18)	6.87	6.69	*d*	3.5	H–C(19)
H–C(5)	6.90	7.10	*s*		
H–C(16)	7.55	7.49	*s*		
H–C(9)	7.84	7.85	*d*	1.4	H–C(8)
H–C(20)	7.85	7.81	*d*	1.4	H–C(19)
H–C(13)	7.87	7.82	*d*	1.4	H–C(12)
H–C(7)	8.21	8.23	*d*	3.5	H–C(8)
H–C(15)	9.48	9.48	*s*		

[a]) Arbitrary numbering according to *Formula* **1a**; for the systematic name, see *Exper. Part.*
[b]) The chemical shifts are given in relation to (D_6)DMSO.
[c]) Determined from 1D spectrum.
[d]) Observed homonuclear ^{1}H,^{1}H connectivities by TOCSY and DQF-COSY.

A comparison of the ^{13}C-NMR spectrum with the results of the DEPT-135 experiment (*Table 2*) indicated nine quarternary signals for each conformer **1a$_1$** and **1a$_2$**. Unequivocal assignment of the quarternary C-atoms could then be successfully achieved by means of heteronuclear multiple-bond/multi-quantum coherence experiments optimized for ^2J(C,H) and ^3J(C,H) coupling constants (*Table 2*).

The furan C-atoms C(7), C(11), and C(18)[1]) of **1a$_1$** resonated in a narrow range of 115.7–116.4 ppm. Heteronuclear correlations of the furan atoms C(7) and C(6) with the olefinic proton H–C(5) were consistent with the furylmethylidene structure proposed in **1a**. This olefinic proton showed further coupling with two quarternary C-atoms resonating at 133.8 (C(4)) and 180.3 ppm (C(3); for **1a$_1$**). In addition to the correlation with H–C(5), the signal at 133.8 ppm showed a cross peak with H–C(21) of the alanine moiety. These data confirm that the amino function of alanine is directly linked to the quarternary C(4). The (*E*)-configuration of the C(4)=C(5) bond was evidenced by NOESY spectroscopy showing a strong NOE effect between H–C(5) and H–C(21). The correlation of H–C(5) with the quarternary C(3) at 180.3 ppm was helpful to assign the carbonyl function at C(3). Due to the magnetic anisotropy of the carbonyl function, the proximate furan H–C(7) being nearly coplanar with the carbonyl function should be strongly deshielded. This assumption is well in line with the experimental data (*Table 1*). Such 'proximity' interactions with carbonyl functions have generally very pronounced effects on the chemical shifts of *peri* protons. This *peri* effect leading to abnormally strong deshielding of a H-atom was also reported for a series of polycyclic ketones [9].

In comparison to the expected chemical shift of ketone C-atoms, the signal of C(3) was highfield shifted. The shielding of this C-atom is obviously due to the electron-donating effect of the amino group of the alanine moiety which is in vinylogous position to the carbonyl function of the proposed β-amino keto structure. HMQC and HMBC Experiments confirmed the incorporation of the amino group of alanine in the β-amino keto system and suggested a 1*H*-pyrrol-3(2*H*)-one structure for **1a**, because H–C(21) of the alanine showed strong correlations to the quarterny C(4) at 133.8 ppm and C(1) at 153.1 ppm. The 1*H*-pyrrol-3(2*H*)-one structure was further corroborated by IR measurements. The C=O streching absorption band at 1653 cm^{-1} in the IR spectrum ($CHCl_3$) of **1a** was well in line with IR data obtained for the 4,5-dimethyl-1*H*-pyrrol-3(2*H*)-one ($\tilde{\nu}$(CO) 1654 cm^{-1} in $CHCl_3$) [10]. The 1*H*-pyrrol-3(2*H*)-one structure of **1a** showed a more zwitterionic character compared with the benzo homologue indoxyl (= 1*H*-indol-3-ol; $\tilde{\nu}$(CO) 1690 cm^{-1} in $CHCl_3$) [11], in which a

Table 2. *Assignment of ^{13}C-NMR Signals* (360 MHz, (D_6)DMSO) *of* **1a**[a])

	δ [ppm][b])		DEPT[c])	Heteronuclear $^1H,^{13}C$ multiple-quantum coherence[d])	
	1a$_1$	**1a**$_2$		*via* ^1J(C,H)	*via* $^{2,3}J$(C,H)
C(22)	17.8	17.1	Me	Me(22)	H–C(21)
C(21)	57.4	57.2	CH	H–C(21)	Me(22)
C(2)	105.7	104.9	C		H–C(15), H–C(16)
C(5)	109.7	110.6	CH	H–C(5)	H–C(7)
C(12)	112.5	112.4	CH	H–C(12)	H–C(11), H–C(13)
C(8)	113.2	113.2	CH	H–C(8)	H–C(7), H–C(9)
C(19)	113.5	113.5	CH	H–C(19)	H–C(18), H–C(20)
C(11)	115.7	115.8	CH	H–C(11)	H–C(12), H–C(13)
C(7)	115.9	116.0	CH	H–C(7)	H–C(5), H–C(8), H–C(9)
C(18)	116.4	116.5	CH	H–C(18)	H–C(16), H–C(19), H–C(20)
C(14)	130.3	130.1	C		H–C(15), H–C(16)
C(4)	133.8	132.7	C		H–C(5), H–C(21)
C(16)	137.7	136.7	CH	H–C(16)	H–C(15), H–C(18)
C(13)	144.1	144.1	CH	H–C(13)	H–C(11), H–C(12)
C(9)	145.5	145.6	CH	H–C(9)	H–C(7), H–C(8)
C(20)	146.2	146.1	CH	H–C(20)	H–C(18), H–C(19)
C(6)	150.7	150.7	C		H–C(5), H–C(7), H–C(8)
C(17)	151.1	151.1	C		H–C(16), H–C(18), H–C(19)
C(10)	151.2	151.2	C		H–C(11), H–C(12)
C(1)	153.1	153.1	C		H–C(11), H–C(21)
C(23)	171.8	172.3	C		H–C(21), Me(22)
C(3)	180.3	180.5	C		H–C(5)
C(15)	193.1	193.1	CH	H–C(15)	H–C(16)

[a]) Arbitrary numbering according to *Formula* **1a**; for the systematic name, see *Exper. Part.*
[b]) The chemical shifts are given in relation to (D_6)DMSO.
[c]) DEPT-135 spectroscopy.
[d]) Assignments based on HMQC (^1J) and HMBC ($^{2,3}J$) experiments.

corresponding zwitterionic structure could only be formed by dearomatization of the benzol ring. In addition, the uneven molecular mass of 419 Da measured by LC/MS, suggested that one N-atom was present in **1a**, also confirming the proposed 1*H*-pyrrol-3(2*H*)-one structure.

The observed ^2J(C,H) coupling between C(17) and H–C(16) as well as the ^3J(C,H) coupling between C(18) and H–C(16) revealed that the 2-position of the furan ring C(17)···C(20) is connected to the olefinic C(16). Because the HMQC experiment showed a correlation with the proton at 9.48 ppm, the most downfield shifted signal at 193.1 ppm was assigned to the C-atom of the aldehyde function. Further correlations indicated that the furan ring C(17)···C(20) must be in the β-position of an α,β-unsatured aldehyde, because no homonuclear coupling could be observed between H–C(15) and H–C(16). However, the α-position should be quarternary, because both, H–C(16) and H–C(15), showed heteronuclear coupling to a quarternary C-atom at 105.7 ppm. This indicates that the α-position of the proposed β-(2-furyl)acrylaldehyde structure is directly linked to C(2) of the pyrrolone moiety. The (*E*)-configuration of C(14)=C(16) was further evidenced by a NOESY experiment revealing a strong NOE effect between the aldehydic H–C(15) and the olefinic H–C(16). Further HMBC experiments confirmed that the quarternary C(1) was directly connected to the third furan ring C(10)···C(13).

2.2. *(E)/(Z)-Equilibrium.* By using reversed-phase HPLC (*RP-18*), the isolated colorant was resolved into two peaks appearing in a ratio of 1:15. The ratio could be shifted from 1:15 to *ca.* 1:6 by acid treatment. To the later eluting compound with a molecular mass of 419, and which was predominating in the NMR spectrum of the mixture, was assigned the (*E,E*)-structure **1a**. The minor, earlier eluting compound revealed the same

molecular mass (HPLC/MS and HPLC/DAD) and a nearly identical UV spectrum as **1a**; however, the λ_{max} was slightly shifted to shorter wavelengths ($\Delta\lambda = 4$ nm). After collection of this HPLC fraction and rechromatography, again the same two peaks appeared, indicating an equilibrium with preference of **1a**.

On the basis of the ^{1}H-NMR data ($CDCl_3$; see *Table 3*) the (*Z*,*E*)-structure **1b** was assigned to the minor isomer.

Table 3. *^{1}H-NMR Chemical Shifts* (360 MHz, $CDCl_3$) *of the* (E,E)*-Isomer* (**1a**) *and the* (Z,E)*-Isomer* (**1b**)[a])

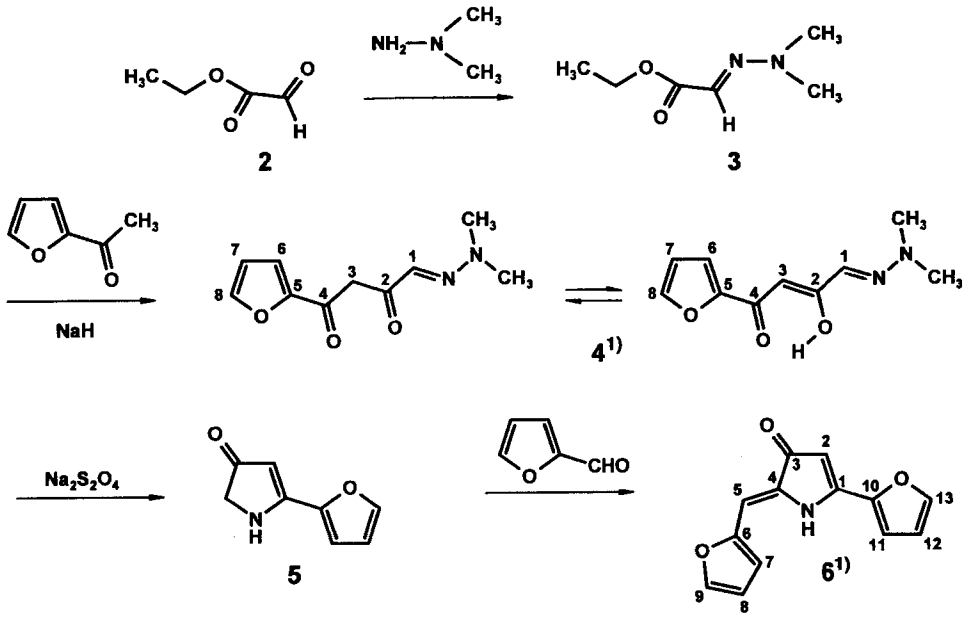
	δ [ppm][b])			
	(*E*,*E*)-Isomer[c])		(*Z*,*E*)-Isomer[c])	
	1a$_1$	**1a$_2$**	**1b$_1$**	**1b$_1$**
Me(22)	1.76	1.66	1.31	1.51
H–C(21)	5.19	5.19	5.26	5.26
H–C(19)	6.30	6.38–6.40	6.38–6.40	6.38–6.40
H–C(8)	6.44	6.44	6.50	6.50
H–C(12)	6.50–6.55	6.50–6.55	6.50–6.55	6.50–6.55
H–C(11)	6.67	6.76	6.92	6.88
H–C(18)	6.72	6.68	6.74	6.78
H–C(5)	6.81	6.82	6.70	6.71
H–C(16)	7.44	7.38	7.41	7.42
H–C(9)	7.39	7.51	7.42	7.45
H–C(13)	7.44	7.55	7.48	7.47
H–C(20)	7.52–7.55	7.52–7.55	7.52–7.55	7.52–7.55
H–C(7)	8.43	8.42	7.11	7.11
H–C(15)	9.48	9.47	9.46	9.44
H–C(23)	10.4–10.7	10.4–10.7	10.4–10.7	10.4–10.7

[a]) Arbitrary numbering according to *Formula* **1a, b**; for systematic names, see *Exper. Part.*
[b]) The chemical shifts are given in relation to $CDCl_3$.
[c]) Assignments based on ^{1}H-NMR, TOCSY, and DQF-COSY measurements.

As observed for **1a**, also for **1b** the ^{1}H-NMR spectrum was split into two spectra sets. Significant differences ($\Delta\delta$) between the two isomers **1a** and **1b** were observed for the chemical shifts of H–C(7), Me(22), and H–C(5)[1]). The most significant $\Delta\delta$ was measured for the furan H–C(7) which appeared upfield-shifted by *ca.* 1.3 ppm for **1b** compared to **1a**. Indeed, the conversion of the (*E*,*E*)- to the (*Z*,*E*)-configuration moves H–C(7) out of the strong deshielding environment caused by the carbonyl function C(3)=O. Also the olefinic H–C(5) of **1b** was more shielded compared with **1a** ($\Delta\delta = 0.11$ ppm for the major conformer). Furthermore, Me(22) of **1b** was upfield shifted by 0.45 and or 0.15 ppm, respectively, for the two conformers. Contrary, the chemical shifts of H–C(15) and H–C(16) of the (*E*)-β-(2-furyl)acryladehyde moiety were only weakly influenced by the configuration of the C(4)=C(5) bond.

2.3. *Synthetic Experiments.* To further confirm the proposed structures, we synthesized the 5-(2-furyl)-2-[(2-furyl)methylidene]pyrrol-3(2*H*)-one (**6**) following the reaction sequence outlined in the *Scheme*. Ethyl glyoxylate dimethylhydrazone (**3**), which was prepared from ethyl glyoxylate (**2**) and dimethylhydrazine in toluene, was reacted with 1-(2-furyl)ethan-1-one *via Claisen* condensation with NaH in dimethylformamide (DMF) yielding the 4-(2-furyl)-2,4-dioxobutanal dimethylhydrazone (**4**). Reduction of the hydrazone with sodium dithionit and subsequent ring closure lead to 5-(2-furyl)-1*H*-pyrrol-3(2*H*)-one (**5**) which was then, without further purification, condensed with furan-2-carbaldehyde affording 1*H*-pyrrol-3(2*H*)-one **6**.

Scheme. *Preparation of 5-(2-Furyl)-2-[(2-furyl)methylidene]-1H-pyrrol-3(2H)-one* (**6**)



Both, the ^{1}H- and ^{13}C-NMR data ($CDCl_3$; see *Exper. Part*) of **6** are well in line with the corresponding three-ring structure of **1a**. Only the signal of H–C(7)[1]) of **6** (7.08 ppm) was strongly highfield-shifted compared with the corresponding furan H-atom of **1a** (8.43 ppm; see *Table 3*). However H–C(7) of **6** resonated close to the signal of H–C(7) of **1b** (7.11 ppm). This indicates a less deshielding environment being well in line with the (*Z*)-configuration of **6**. The preference of the (*Z*)-configuration of **6** is undoubtedly due to the less steric hindrance, whereas in **1a** the voluminous alanine moiety favors the (*E*)-configuration. This was also confirmed by molecular-mechanics calculations (data not shown).

2.4. *Conformational Studies.* Both, **1a** and **1b**, showed the signals of two isomers (**1a**$_1$/**1a**$_2$ or **1b**$_1$/**1b**$_2$) in the NMR spectra. To gain a more detailed insight into, whether the splitting of the spectra is due to two conformations of **1a** and **1b** or is alternatively caused by a further (*E*)/(*Z*)-equilibrium, *e.g.*, of the C(14)=C(16) bond, we measured NOE effects. Since **1a** was the predominant compound in the mixture **1a**/**1b** and, in addition, **1b** could not be isolated without rapid conversion into **1a**, the conformational studies were performed with **1a** as the example. The conformation of **1a** was deduced by two-dimensional NOESY experiments (*Table 4*). Because the 2D-NOESY cross peaks H–C(5)/Me(22), H–C(5)/H–C(21), and also H–C(15)/H–C(16)[1]) showed nearly the same intensity for both isomers **1a**$_1$ and **1a**$_2$, it is obvious that the splitting of the set of spectra of **1a** are not be caused by a further (*E*)/(*Z*)-isomerization, but must indicate the existence of two conformations.

Besides a strong NOE between Me(22) and the olefinic H–C(5) which is consistent with the (*E*)-configuration of the C(4)=C(5) bond in **1a**, a through-space connectivity was observed between Me(22) and H–C(18). The latter NOE is outlined in the left structure of *Fig. 1* showing the energy-minimized conformers **1a**$_1$ and **1a**$_2$, which were

Table 4. *^1H, ^1H-NOE Observations in* **1a** (600 MHz, (D_6)DMSO)[a])

	Observed NOE (sign)	
	via NOESY	*via* ROESY
Me(22)	H–C(5)(–), H–C(18)(–)	H–C(5)(+), H–C(11)(+), H–C(18)(+)
H–C(21)	H–C(5)(–), H–C(11)(+)	H–C(5)(+), H–C(11)(+), H–C(18)(+)
H–C(19)		H–C(7)(+)
H–C(8)		
H–C(12)		
H–C(11)	H–C(21)(+)	H–C(15)(+), H–C(16)(+), H–C(21)(+), Me(22)(+)
H–C(18)	H–C(7)(–), H–C(16)(–)	H–C(7)(+), H–C(16)(+), H–C(21)(+), Me(22)(+)
H–C(5)	H–C(21)(–), H–C(22)(–)	H–C(21)(+), Me(22)(+)
H–C(16)	H–C(11)(–), H–C(15)(–), H–C(18)(–)	H–C(11)(+), H–C(15)(+), H–C(18)(+)
H–C(9)		
H–C(20)		
H–C(13)		
H–C(7)	H–C(18)(–)	H–C(18)(+), H–C(19)(+)
H–C(15)	H–C(16)(+)	H–C(11)(+), H–C(16)(+)

[a]) Arbitrary numbering according to Formula **1a**; for systematic name, see *Exper. Part.*

suggested by molecular-mechanics modelling. Molecular-mechanics calculations revealed an interproton distance between Me(22) and H–C(18) of 3.8 Å. Beside the NOE with Me(22), the ring proton H–C(18) showed a further NOE with H–C(7) which is consistent with the proposed orientation of the (*E*)-β-(2-furyl)acrylaldehyde moiety in **1a**. An interproton distance of 3.0 Å was calculated by molecular-mechanics modelling.

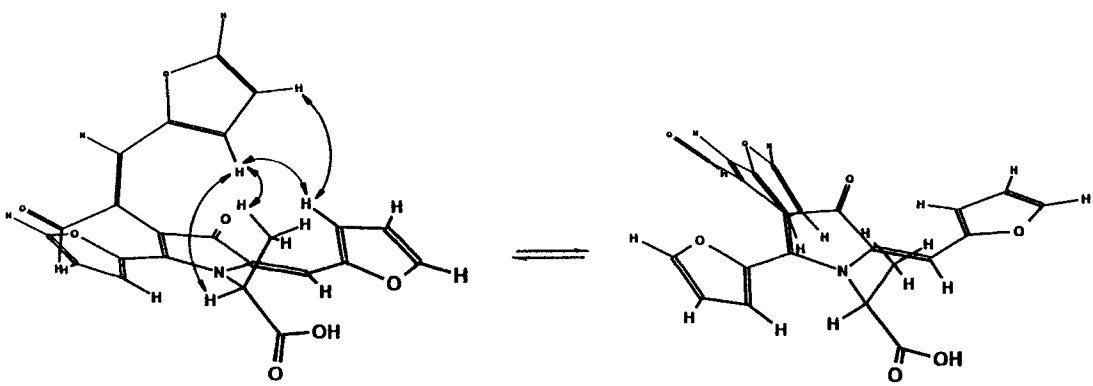


Fig. 1. *Conformation of atropisomers* $\mathbf{1a}_1$ *and* $\mathbf{1a}_2$ *of* (S)*-4-[(*E*)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid*

The NOEs listed in *Table 4* show that some NOEs have a negative (extreme narrowing) and some positive (slow tumbling) signs. However, it is known [12] [13] that in medium-size molecules, when the correlation time τ_c approaches the inverse of the *Larmor* frequency of the protons, cross-peak intensities are often close to zero. Some NOEs might, therefore, not be detected by the NOESY experiments. To overcome this problem, cross-relaxation in the rotating frame, measured by a ROESY experiment (under spin-locked conditions), can be used, because molecular reorientation rates are fast compared to the effective *Larmor* frequency in the rotating frame, resulting in only positive NOEs [12] [13].

The ROESY experiment (*Table 4*) confirmed the results of the NOESY, as shown in *Fig. 2*, *e.g.*, for the NOEs H–C(5)/H–C(21), H–C(5)/Me(22), H–C(18)/Me(22), and H–C(11)/H–C(21). However, in addition to the

NOESY results, the ROESY experiment revealed some more through-space connectivities (*Table 4*). The NOE between H–C(18) and H–C(21), (see *Fig. 2*), or between H–C(7) and H–C(19) confirmed the conformation proposed in *Fig. 1*.

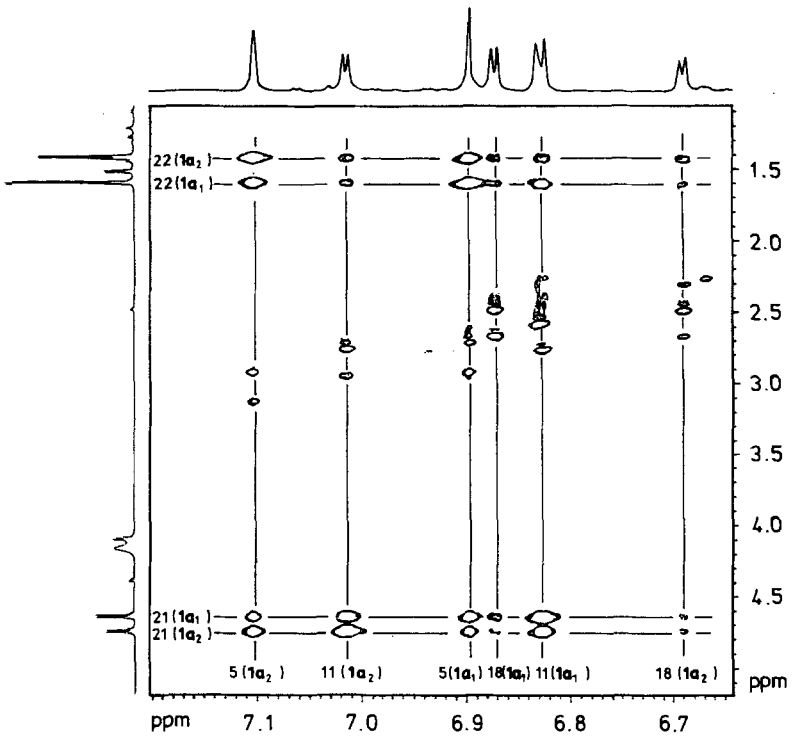


Fig. 2. *Selected region of the contour plot of the 2D-ROESY experiment of* **1a** *in (D$_6$)DMSO.* A 4-kHz spin-lock field was used during the 220 ms mixing period. Arbitrary numbering according to *Formula* **1a**.

Besides the strong NOEs H–C(11)/H–C(21) as well as H–C(11)/Me(22), weak NOEs between H–C(11) and H–C(15) as well as H–C(16) were observed. These correlations indicated a lower population of the corresponding rotamer of the furan ring C(10)···C(13) and confirmed the orientation of the (*E*)-β-(2-furyl)acrylaldehyde branch in **1a**. The same was found for the furan ring C(17)···C(20), because a weak NOE between H–C(16) and H–C(18) was observed.

The cross peaks in *Fig. 2*, which cannot be assigned to H-atoms in the F_2 dimension, are part of the double-quantum diagonal, a typical artifact of the ROESY experiment. However, beside the NOEs measured in each single conformer, *e.g.* H–C(5), (**1a$_1$**)/H–C(21) (**1a$_1$**) or H–C(5) (**1a$_2$**)/H–C(21) (**1a$_2$**), also off-diagonal exchange cross peaks, *e.g.* H–C(5) (**1a$_1$**)/H–C(21) (**1a$_2$**) or H–C(5) (**1a$_2$**)/H–C(21) (**1a$_1$**) could be observed with somewhat lower intensities (*Fig. 2*). The detection of, *e.g.*, the exchange cross peak H–C(5) (**1a$_1$**)/H–C(21) (**1a$_2$**) indicates that a conversion of **1a$_1$** to **1a$_2$** during the mixing period τ_m of 600 ms (NOESY) or 220 ms (ROESY) has transferred magnetization components of the precession frequency of H–C(5) (**1a$_1$**) to a transition of the frequency of H–C(21) (**1a$_1$**). The appearence of off-diagonal cross peaks in the 2D NMR spectroscopy during exchange processes in molecular systems was reported earlier by *Jeener et al.* [14] and *Macura et al.* [15].

The NOE data indicate that the β-(2-furyl)acrylaldehyde moiety in **1a** can not be coplanar with, but must come out of the planarity of the 5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-1*H*-pyrrol-3(2*H*)-one system. Dihedral angels of *ca.* 40° between the coplanar three-ring system and the (*E*)-β-(2-furyl) acrylaldehyde branch were calculated by molecular-mechanics simulation. Because, depending on the conformer, the β-(2-furyl)acrylaldehyde branch is either above or below the plane of the three-ring system, a free rotation of the alanine moiety around the N–C(21) bond is possible in both conformations; corresponding NOEs, *e.g.* H–C(18)/H–C(21) or H–C(18)/Me(22), were

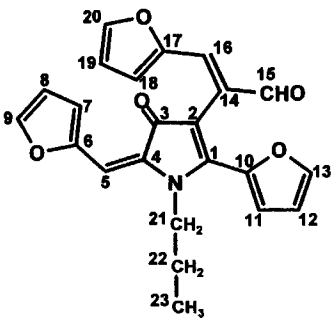
observed for both $\mathbf{1a}_1$ and $\mathbf{1a}_2$. To enable the flipping of the β-(2-furyl)acrylaldehyde branch through the plane of the three-ring system, an energy barrier has to be overcome resulting in the separate conformers $\mathbf{1a}_1$ and $\mathbf{1a}_2$ at room temperature. Due to the resulting chiral axis in the molecule, these conformers are atropisomers. Because the chiral center of the L-alanine moiety exists as a further symmetry element, the two atropisomers are, therefore, also diastereoisomers, leading to 'diastereotopic' splitting on the typical chemical-shift time scale of the NMR experiment.

2.5. *Influence of the Temperature.* Because exchange processes were observed during 2D NOE spectroscopy, the energy barrier for the conversion of both conformers $\mathbf{1a}_1$ and $\mathbf{1a}_2$ should be rather low. Therefore, on rising the temperature, a coalescence of the two signal sets in the ^{1}H-NMR spectrum might be expected. ^{1}H-NMR Spectra were measured at different temperatures between 300 and 340 K, the upper temperature limit being imposed by the boiling point of the solvent (D_6)DMSO in the vacuum degassed NMR tube. Increasing the temperature led indeed to a significant decrease of the $\Delta\delta$ values of the two conformers. As the conversion of $\mathbf{1a}_1$ and $\mathbf{1a}_2$ is very rapid on the typical chemical-shift time scale of the NMR experiment, a unique averaged resonance was observed at 340 K for H–C(7), H–C(8), H–C(9), H–C(12), H–C(13), H–C(15), H–C(16), H–C(19), and H–C(20)[1]). The coalescence was reversible, cooling the NMR tube to 300 K resulting again in a splitted spectra set. As an example, the influence of the temperature on the chemical shift of H–C(7) of both atropisomers is shown in *Fig. 4*. At 300 K, the *d*s of the two atropisomers $\mathbf{1a}_1$ and $\mathbf{1a}_2$ are separated; on raising the temperature, these *d*s run together to give, at 320 K, a broad unresolved signal and, at 340 K, a *d* indicating total coalescence. The data show that at higher temperatures, the 'diastereotopic splitting' reaches zero and indicate that, due to the rapid flipping of the (*E*)-β-(2-furyl)acrylaldehyde branch, only a single symmetry element, the chiral center of the L-alanine, remains. The resulting enantiomeric atropisomers can not be differentiated by NMR spectroscopy.

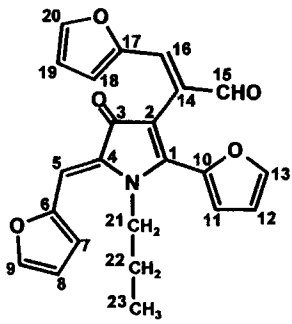
2.6. *Replacement of the* L-*Alanine by a Propylamine Moiety: Synthesis of* **7a**, **7b**. According to the data of *Sect. 2.4* and *2.5*, replacement of the L-alanine moiety in **1** with an achiral amino compound should result in a product not showing 'diastereotopic splitting' of its ^{1}H-NMR spectrum. To check this hypothesis, furan-2-carboxaldehyde was reacted with propylamine affording 2-furyl-α,5-bis[(*E*)-(2-furyl)methylidene]-4,5-dihydro-4-oxo-1-propyl-1*H*-pyrrol-3-acetaldehyde (**7a**) as the major isomer. The NMR data (see *Exper. Part*) revealed the presence of the minor isomer **7b** with (*Z*,*E*)-configuration.

The ratio **7a**/**7b** of 5:1, as compared with the ratio **1a**/**1b**, of 15:1, is well in line with the less steric hindrance of the propyl moiety, and the unsplitted NMR spectra are consistent with the lack of achiral center in this moiety.

2.7. *Chromophoric System.* The UV/VIS spectrum of **1a** in H_2O (pH 7.0) showed three maxima, the most intense at 414 nm, one at 330 nm, and a broad one with weak intensity at 480 nm. The fact that the (*E*)-β-(2-furyl)acrylaldehyde moiety of **1a** is not coplanar with the planar three-ring system (see above) should be reflected in its UV/VIS spectra. Indeed, comparison with the data of the synthetic 1*H*-pyrrol-3(2*H*)-one **6** (λ_{max} 406 nm) showed that the λ_{max} of **1a** (414 nm) was only slightly shifted to higher wavelengths confirming the 5-(2-furyl)-2-[(2-furyl)methylidene]-1*H*-pyrrol-3(2*H*)-one structure as the main chromophore in **1a**. Moreover, the UV maximum of (*E*)-β-(2-furyl)acrylaldehyde (λ_{max} 324 nm) is in the range of the second UV maximum of **1a** (330 nm in H_2O). This indicates that the β-(2-furyl)acrylaldehyde moiety of **1a** is mostly



7a[1)]



7b[1)]

Table 5. *Influence of the Temperature on the* ^{1}*H-NMR Chemical Shifts* (600 MHz, (D_6)DMSO) *of the Conformers* $\mathbf{1a}_1$ *and* $\mathbf{1a}_2$[a])

	δ [ppm][b]) at			
	300 K		340 K	
	$\mathbf{1a}_1$	$\mathbf{1a}_2$	$\mathbf{1a}_1$	$\mathbf{1a}_2$
Me(22)	1.60	1.43	1.54	1.48
H–C(21)	4.64	4.75	4.64	4.65
H–C(19)	6.55	6.58	6.53	6.53
H–C(8)	6.61–6.63	6.61–6.63	6.57–6.58	6.57–6.58
H–C(12)	6.61–6.63	6.61–6.63	6.57–6.58	6.57–6.58
H–C(11)	6.83	7.02	8.82	6.93
H–C(18)	6.87	6.69	6.81	6.72
H–C(5)	6.90	7.10	6.97	7.11
H–C(16)	7.55	7.49	7.47	7.47
H–C(9)	7.84	7.85	7.77–7.79	7.77–7.79
H–C(20)	7.85	7.81	7.77–7.79	7.77–7.79
H–C(13)	7.87	7.82	7.77–7.79	7.77–7.79
H–C(7)	8.21	8.23	8.21	8.21
H–C(15)	9.48	9.48	9.48	9.48

[a]) Arbitrary numbering according to *Formula* **1a**; for systematic name, see *Exper. Part.*
[b]) The chemical shifts are given in relation to internal (D_5)DMSO.

not in conjugation with the three-ring system and should, therefore, play only a minor role in generating the red-orange color. The less intense and broad UV/VIS maximum of **1a** at 480 nm might be due to the short-time extension of the chromophoric system at the moment of coplanarity of the molecule during the flipping of the β-(2-furyl)acrylaldehyde branch.

3. Conclusion. – The identification of colored compounds formed from *Maillard*-type model reactions, *e.g.*, from furan-2-carbaldehyde and L-alanine, provides useful informations to extend the knowledge on their chromophores and will help to understand the type of reactions leading to color development during thermal processing of foods. In

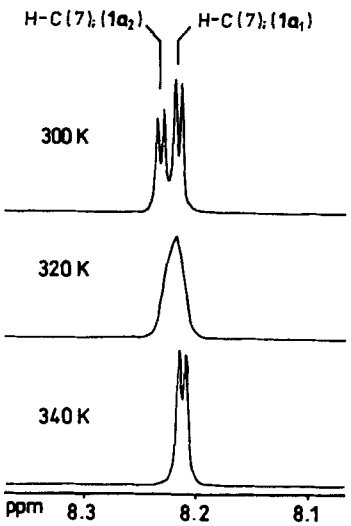


Fig. 3. *Influence of the temperature on the ^{1}H-NMR chemical shift of H–C(7) of the conformers* **1a**$_1$ *and* **1a**$_2$. Arbitrary numbering according to *Formula* **1a**.

addition to the low-molecular-weight colorants, also colored compounds with high molecular weights are reported to be formed during food processing, but nothing is known on the chemical nature of these so-called melanoidins. An incorporation of low molecular weight colorants in biopolymers might be involved in the formation of these melanoidins: *e.g.*, the N-terminal amino acid of a protein might react with furan-2-carbaldehyde in the same way as described in the present investigation for the free amino acid resulting in the formation of a colored protein-linked 1*H*-pyrrol-3(2*H*)-one. To extend the knowledge about the structure and formation of colored compounds formed during nonenzymatic browing reactions, is, therefore, an important task for the future.

Experimental Part

General. The following compounds were obtained commercially: furan-2-carboxaldehyde, 1-(2-furyl)ethan-1-one, (*E*)-3-(2-furyl)prop-2-enal, ethyl glyoxylate in toluene, dimethylhydrazine, NaH, propylamine, CF_3COOH (*Aldrich*, Steinheim, FRG), sodium dithionit (*Merck*, Darmstadt, FRG), and L-alanine (*Lancaster*, Mühlheim, FRG). AcOEt, pentane, Et_2O, DMF, EtOH, CH_2Cl_2, and MeOH were HPLC-grade (*Aldrich*, Steinheim, FRG). (D_6)DMSO and $CDCl_3$ were from *Isocom* (Landshut, FRG). Column chromatography (CC): silica gel *60* (*Merck*, Darmstadt, FRG); H_2O-cooled glass column (40 × 2 cm). Gas chromatography/mass spectroscopy (GC/MS): type-*5160* gas chromatograph (*Fisons Instruments*, Mainz, FRG), *SE-54* capillary (30 m × 0.32 mm, 0.25 μm; *J & W Scientific*, *Fisons Instruments*, Mainz, FRG) coupled with an MD-800 mass spectrometer (*Fisons Instruments*, Mainz, FRG); sample application (0.5 μl) by on-column injection at 40°. HPLC/Diode-array detector (HPLC/DAD): HPLC system (*Rheodyne* injector 100-μl loop, gradient mixer *M800*, 2 pumps type *422*) and DAD (typ *440*) from *Kontron* (Eching, FRG); *RP-18* material (*ODS-Hypersil*, 5 μm, 10 nm, *Shandon*, Frankfurt, FRG) for anal. (250 × 4.6 mm, flow rate 0.8 ml/min) and prep. HPLC (250 × 10 mm, flow rate 1.8 ml/min). HPLC/MS: anal. HPLC coupled with an *LCQ-MS* (*Finnigan MAT GmbH*, Bremen, FRG); gradient MeCN/0.1% aq. CF_3COOH soln. 30:70 → MeCN within 15 min. UV: spectrometer *U-2000* (*Colora Messtechnik GmbH*, Lorch, FRG); λ_{max} in nm, ε in $1mol^{-1}cm^{-1}$. IR: spectrometer *288 B* (*Perkin-Elmer*, Überlingen, FRG); in cm^{-1}. Molecular mechanics calculations were performed using a MM3 force field (Alchemy III).

NMR Spectroscopy. Bruker-AC-200 spectrometer, at 297 K, for DQF-COSY, TOCSY, HMQC, HMBC, and HMQC-DEPT experiments; *Bruker-AM-360* spectrometer, at 297 K, for ^{1}H, ^{13}C, and DEPT-135 experiments;

Bruker AMX-600 spectrometer, at 300 K, for ^{1}H, HMQC, HMBC, NOESY, and ROESY experiments and for the temperature course. For HMQC and HMBC measurements, the protons connected to ^{12}C atoms were suppressed by a BIRD pulse according to *Bax* and *Summers* [16]. For NOESY and ROESY experiments, the sample was freed from O_2 by a three-step-pump-freeze-thaw cycle. Evaluation of the experiments were done with 1D- and 2D-WIN-NMR as well as UX-NMR software (*Bruker*). Sample (*ca.* 10 mg), in $CDCl_3$ or (D_6)DMSO in a *Wilmad-535-PP* tube; chemical shifts in ppm were measured from residual $CHCl_3$ (7.24 ppm) or (D_5)DMSO (2.49 ppm) in the proton dimension and with the C-signal of $CHCl_3$ (78.0 ppm) or (D_6)DMSO (39.5 ppm) in the C-dimension. ^{1}H-NMR: *AM 360*: transmitter frequency 360.1299991 MHz, spectral width 7246.377 Hz, repetition time 3.2 s, recorded with 64 K data points, 256 scans; *AMX 600*: transmitter frequency 600.1423211 MHz, spectral width 10204 Hz, pulse length 10.4 μs, repetition time 2.5 s, 64 scans; processing was done by multiplication with a *Lorentz-Gaussian* function prior to transformation. ^{13}C-NMR: transmitter frequency 90.56 MHz, spectral width 25000 Hz, pulse length 10.4 μs, recorded with 64 K data points, repetition time 2.5 s, 1-Hz line broadening; processing was done by multiplication with a *Lorentz-Gaussian* function prior to transformation. COSY [17] [18]: a phase-sensitive double-quantum-filtered COSY was performed (DQF-COSY), relaxation delay 3 s, 2 K data-points in F_2 and 400 experiments in F_1, 32 scans, 2 dummy scans, spectral width 2000 Hz and resolution 2 Hz/point in both dimensions; sine-bell multiplication gives 1K × 1K complex points. TOCSY [19] [20]: MLEV-17 mixing sequence τ_m = 8 ms with 2.5 ms trim pulses; 2 K data points in F_2 and 200 experiments in F_1, 4 scans, spectral width 2000 Hz, in both dimensions. HMQC [21] [22]: (^{1}H) transmitter frequency 600.1423211 MHz, (^{1}H) spectral width 7246.38 Hz, (^{13}C) transmitter frequency 150.9210611 MHz, (^{13}C) spectral width 29411.8 Hz, DW 86 μs, 8 scans, $TD = 4k(F_2) \cdot 1k(F_1)$, D_1 = 240 ms, D_0 = 3 μs, D_2 = 3.57 ms, AQ = 176 ms, D_4 = 236 ms, hl_1 = 3 dB, $P_1(HL_1)$ = 10.4 μs, P_2 = 20.8 μs, P_3 = 23 μs, P_4 = 46 μs (90°/180°-carbon impuls), ^{13}C-transmitting power 5 dB/300 W. Processing was done by multiplication with a 90°-shifted squared sine bell in both dimensions prior to two dimensional *Fourier* transformation, followed by zero filling in F_1, gives a matrix of 4 K(F_2) × 1 K(F_1) data points. HMBC [16]: (^{1}H) transmitter frequency 600.1423211 MHz, (^{1}H) spectral width 7246.38 Hz, (^{13}C) transmitter frequency 150.9210611 MHz, (^{13}C) spectral width 29411.8 Hz, DW = 86 μs, 8 scans, $TD = 4k(F_2) \cdot 512(F_1)$, D_1 = 1.5s, D_0 = 3 μs, D_2 = 3.57 ms, AQ = 0.283 ms, evolution time 60 ms, hl_1 = 3 dB, $P_1(HL_1)$ = 10.4 μs, P_2 = 20.8 μs, P_3 = 23 μs, P_4 = 46 μs. Processing was done by multiplication with a 90° shifted squared sine bell in both dimensions prior to two dimensional *Fourier* transformation, followed by zero filling in F_1 and magnitude calculation in F_2, gives a matrix of 4 K(F_2) × 1 K(F_1) data points. NOESY [23]: transmitter frequency 600.1423211 MHz, spectral width 7246.38 Hz, DW = 69 μs, DS = 8, $TD = 8k(F_2) \cdot 1k(F_1)$, D_1 = 2.5s, D_0 = 2 μs, hl_1 = 3 dB, $P_1(HL_1)$ = 10.4 μs, τ_m = 600 ms. Processing was done by multiplication with a 90°-shifted squared sine bell in both dimensions prior to two dimensional *Fourier* transformation, followed by zero filling in F_1, gives a matrix of 4 K(F_2) × 1 K(F_1) data points. ROESY [24–26]: transmitter frequency 600.1423211 MHz, spectral width 7246.38 Hz, DW = 69 μs, DS = 16, $TD = 8k(F_2) \cdot 1k(F_1)$, D_1 = 2.5s, D_0 = 2 μs, AQ = 0565 ms, hl_1 = 3 dB, $P_1(HL_1)$ = 10.4 μs, p5 = 2 μs, τ_m = 220 ms. Spinlock 4 kHz (pulsed spinlock) with 2 μs pulses in a distance of 10 μs. Processing was done by multiplication with a 90°-shifted squared sine bell in both dimensions prior to two-dimensional *Fourier* transformation, followed by zero filling in F_1, gives a matrix of 4 K(F_2) × 1 K(F_1) data points.

(S)-4-[(E)-1-Formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(E)-(2-furyl)methylidene]-2,3-dihydro-α-methyl-3-oxo-1H-pyrrole-1-acetic Acid (**1a**) *and Its 2-[(Z)-(2-Furyl)methylidene] Isomer* **1b**. Isomers **1a** and **1b** were isolated from a heated aq. soln. of furan-2-carbaldehyde and L-alanine by extraction with AcOEt and CC (silica gel) and purified by anion exchange chromatography and, in addition, by semi-prep. TLC (silica gel) and prep. reversed-phase HPLC [7]. UV (H_2O, pH 7.0): 330, 414 nm (2.9 · 10^4), 480. IR ($CHCl_3$): 3016, 2975, 1653, 1469, 1046. ^{1}H-NMR (600 MHz, (D_6)DMSO; 360 MHz, $CDCl_3$): *Tables 1* and *3*. ^{13}C-NMR (360 MHz, (D_6)DMSO): *Table 2*. HPLC/MS: 420 (100, $[M + 1]^+$), 442 (24, $[M + Na]^+$), 392 (20), 319 (13)

Ethyl (Dimethylhydrazono)acetate (**3**). Dimethyl hydrazine (20.5 mmol) was added to a stirred soln. of ethyl glyoxylate hydrate (20.0 mmol) in toluene (40 ml). After 2 h at r.t., the solvent was evaporated affording (92%). Pale yellow oil. ^{1}H-NMR (360 MHz, $CDCl_3$): 1.31 (*t*, ^3J = 7.07, *Me*CH_2O); 3.14 (*s*, Me_2N); 4.23 (*q*, ^3J = 7.07, MeCH_2O); 6.37 (*s*, CH=N). ^{13}C-NMR (360 MHz, $CDCl_3$): 13.5 (*Me*CH_2O); 41.6 (Me_2N); 59.2 (MeCH_2O); 116.3 (CH=N); 164.3 (COO). GC/MS(EI): 144 (100), 99 (82), 74 (82), 44 (81), 71 (80), 42 (77), 43 (72), 102 (68), 103 (63), 115 (32), 57 (31)

4-(2-Furyl)-2,4-dioxobutanal Dimethylhydrazone (**4**). Following a procedure of [27] with some modifications, NaH (30 mmol) was added in small portions to a stirred soln. of **3** (10 mmol) and 1-(2-furyl)ethan-1-one (11 mmol) in DMF (8 ml) under Ar. After 4 h at r.t., unreacted NaH was quenched by addition of MeOH. The mixture was then diluted with H_2O (100 ml) and washed with Et_2O (50 ml). The org. layer was discarded, the pH of the aq. phase adjusted to 7.0 with HCl soln. (1 mol/l), and the aq. layer then extracted with AcOEt (5 × 50 ml). After

drying (Na_2SO_4), the org. layer was evaporated, the residue submitted to CC (silica gel conditioned with pentane/Et_2O 1:1, pentane/Et_2O 1:1 (150 ml) and then 3:7 (200 ml)), and the product crystallized: **4** (71%). Yellow needles. A 1:1.5 ratio of the diketo/keto enol form was calculated from the ^{1}H-NMR.

Diketo isomer: ^{1}H-NMR (360 MHz, $CDCl_3$)[1]): 3.10 (*s*, Me_2N); 4.21 (*s*, CH_2(3)); 6.54 (*dd*, *J*(7,6) = 3.54, *J*(7,8) = 1.77, H–C(7)); 6.62 (*s*, H–C(1)); 7.24 (*dd*, *J*(6,7) = 3.54, *J*(6,8) = 0.88, H–C(6)); 7.57 (*dd*, *J*(8,7) = 1.77, *J*(8,6) = 0.88, H–C(8)). ^{13}C-NMR (360 MHz, $CDCl_3$)[1]): 42.6 (Me_2N); 47.9 (C(3)); 112.3 (C(7)); 117.7 (C(6)); 126.6 (C(1)); 146.4 (C(8)); 152.7 (C(6)); 183.8 (C(4)); 191.8 (C(2)). GC/MS(EI): 95 (100), 44 (72), 137 (68), 110 (41), 71 (40), 166 (37), 208 (36), 69 (36), 59 (30), 43 (30), 167 (25).

Keto enol isomer: ^{1}H-NMR (360 MHz, $CDCl_3$)[1]): 3.19 (*s*, Me_2N); 6.52 (*dd*, *J*(7,6) = 3.54, *J*(7,8) = 1.77, H–C(7)); 6.57 (*s*, H–C(3)); 6.59 (*s*, H–C(1)); 7.07 (*dd*, *J*(6,7) = 3.54, *J*(6,8) = 0.88, H–C(6)); 7.54 (*dd*, *J*(8,7) = 1.77, *J*(8,6) = 0.88, H–C(8)). ^{13}C-NMR (360 MHz, $CDCl_3$)[1]): 42.6 (Me_2N); 92.1 (C(3)); 112.2 (C(7)); 113.8 (C(6)); 124.3 (C(1)); 145.1 (C(8)); 150.4 (C(6)); 170.6 (C(4)); 185.2 (C(2)). GC/MS(EI): 95 (100), 44 (82), 110 (61), 71 (56), 69 (55), 137 (54), 43 (50), 166 (45), 208 (44), 57 (44), 191 (18).

*5-(2-Furyl)-1*H*-pyrrol-3(2*H*)-one* (**5**). Using a reduction procedure of [27], a mixture of **4** (5.0 mmol) and sodium dithionit (15 mmol) in EtOH/H_2O 2:1 (*v*/*v*; 20 ml) was refluxed for 20 min, while being vigorously stirred. The suspension was then filtered, the filtrate diluted with H_2O (100 ml) and extracted with CH_2Cl_2 (6 × 30 ml), the org. phase dried (Na_2SO_4), and **5** (64%) analyzed by GC/MS. Measuring an NMR spectrum was not successful, because **5** polymerized rapidly on further concentration of the soln. GC/MS(EI): 149 (100), 92 (73), 120 (35), 63 (33), 93 (21), 64 (20), 65 (19).

*5-(2-Furyl)2-[(2-furyl)methylidene]-1*H*-pyrrol-3(2*H*)-one* (**6**). A soln. of furan-2-carboxaldehyde (3.2 mmol) in EtOH (20 ml) was added to a soln. of **5** (3.1 mmol) in CH_2Cl_2 (100 ml). CH_2Cl_2 was then destilled off *in vacuo*, and the remaining EtOH soln. was maintained in the dark for 3 h at r.t. The solvent was then evaporated and the product isolated by CC on silica gel conditioned with toluene, toluene (200 ml), toluene/AcOEt 9:1 (400 ml) and then 8:2 (200 ml): **6** (94%). Red-orange oil. UV (H_2O, pH 7.0): 406 (2.3 · 10^4). IR ($CHCl_3$): 1670, 1626, 1589. ^{1}H-NMR (360 MHz, $CDCl_3$)[1]): 5.65 (*s*, H–C(2)); 6.57 (*s*, H–C(5)); 6.72 (*dd*, *J*(8,7) = 3.53, *J*(8,9) = 1.33, H–C(8)); 6.82 (*dd*, *J*(12,11) = 3.53, *J*(12,13) = 1.33, H–C(12)); 7.08 (*d*, *J*(7,8) = 3.53, H–C(7)); 7.60 (*d*, *J*(11,12) = 3.53, H–C(11)); 7.93 (*d*, *J*(9,8) = 1.33, H–C(9)); 8.06 (*d*, *J*(13,12) = 1.33, H–C(13)); 9.44 (*bs*, NH); ^{13}C-NMR (360 MHz, $CDCl_3$)[1]): 96.2 (C(2)); 100.4 (C(5)); 113.0 (C(8)); 113.1 (C(12)); 115.1 (C(7)); 115.6 (C(11)); 132.6 (C(4)); 144.8 (C(10)); 145.5 (C(9)); 147.0 (C(13)); 150.0 (C(6)); 154.8 (C(1)); 185.2 (C(3)). GC/MS(EI): 227 (100), 92 (34), 99 (18), 170 (18), 173 (16), 199 (15), 226 (15), 228 (14), 63 (14), 52 (11), 142 (9).

*2-Furyl-α,5-bis[(*E*)-(2-furyl)methylidene]-4,5-dihydro-4-oxo-1-propyl-1*H*-pyrrol-3-acetaldehyde* (**7a**) *and Its 5-[(*Z*)-(2-Furyl)methylidene] Isomer* **7b**. Furan-2-carbaldehyde (0.2 mol) and propylamine (0.2 mol) were reacted in 0.5M phosphate buffer (pH 7.0; 200 ml) for 1 h at 70°. After cooling to r.t., the mixture was extracted with Et_2O (3 × 50 ml), the org. layer washed with 0.1N aq. HCl (2 × 20 ml), dried (Na_2SO_4), and then concentrated to *ca.* 100 ml. After distillation at 50° 0.04 mbar, the remaining residue was taken up in Et_2O (10 ml) and then an aliquot subfractionated by CC (pentane-conditioned silica gel, pentane/Et_2O 8:2 (600 ml), 6:4 (400 ml), 5:5 (200 ml), and 4:6 (400 ml)). The fraction obtained with pentane/Et_2O 4:6 containing the product was further fractionated by prep. TLC (silica gel (20 × 20 cm; 0.5 mm; *Merck*, Darmstadt, FRG), pentane/Et_2O 1:1). The red band with R_f = 0.1 was extracted with MeOH (50 ml) and the concentrated extract submitted to reversed-phase prep. HPLC (MeOH/H_2O 1:1 → MeOH within 40 min). The fraction collected between 21.0 and 22.0 min was diluted with H_2O and extracted with Et_2O and the extract evaporated: 87 mg of **7a**/**7b** 5:1. Deep red solid. UV/VIS (H_2O, pH 7.0): 330, 412 (2.7 · 10^4), 480. IR ($CHCl_3$): 1654. HPLC/MS: 390 (100, $[M + 1]^+$), 802 (21, $[2 \times M + Na]^+$), 362 (8).

(*E*,*E*)-Isomer **7a**: ^{1}H-NMR (360 MHz, (D_6)DMSO)[1]): 0.87 (*t*, *J*(23,22) = 7.52, Me(23)); 1.66 (*m*, *J*(22,23) = 7.52, *J*(22,21) = 7.52, CH_2(22)); 4.04 (*t*, *J*(21,22) = 7.52, CH_2(21)); 6.61–6.69 (3*dd*, *J* = 3.54, 1.77, 1 H each, H–C(8), H–C(12), H–C(19)); 6.74 (*d*, *J*(18,19) = 3.54, H–C(18)); 6.84 (*d*, *J*(11,12) = 3.54, H–C(11)); 6.97 (*s*, H–C(5)); 7.60 (*s*, H–C(16)); 7.83 (*d*, *J*(20,19) = 1.77, H–C(20)); 7.95 (*d*, *J*(9,8) = 1.77, H–C(9)); 7.96 (*d*, *J*(13,12) = 1.77, H–C(13)); 8.29 (*d*, *J*(7,8) = 3.54, H–C(7)); 9.54 (*s*, H–C(15)). ^{13}C-NMR (360 MHz, (D_6)DMSO)[1]): 10.8 (C(23)); 21.7 (C(22)); 45.3 (C(21)); 105.1 (C(2)); 108.2 (C(5)); 112.5 (C(12)); 113.0 (C(8)); 113.1 (C(19)); 116.1 (C(11)); 116.3 (C(7)); 116.9 (C(18)); 129.4 (C(14)); 133.7 (C(4)); 137.3 (C(16)); 145.7 (C(13)); 146.2 (C(9)); 146.4 (C(20)); 150.3 (C(6)); 150.3 (C(10)); 150.8 (C(17)); 151.0 (C(1)); 179.9 (C(3)); 192.9 (C(15)).

(*Z*,*E*)-Isomer **7b**: ^{1}H-NMR (360 MHz, (D_6)DMSO)[1]): 0.70 (*t*, *J*(23,22) = 7.52, Me(23)); 1.66 (*m*, *J*(22,23) = 7.52, *J*(22,21) = 7.52, CH_2(22)); 4.14 (*t*, *J*(21,22) = 7.52, CH_2(21)); 6.61–6.69 (3*dd*, *J* = 3.54, *J* = 1.77, 1 H each, H–C(8), H–C(12), H–C(19)); 6.77 (*d*, *J*(18,19) = 3.54, H–C(18)); 6.93 (*d*, *J*(11,12) = 3.54, H–C(11)); 6.79 (*s*, H–C(5)); 7.61 (*s*, H–C(16)); 7.83 (*d*, *J*(22,21) = 1.77, H–C(20)); 8.05 (*d*, *J*(9,8) = 1.77, H–C(9)); 7.96 (*d*, *J*(13,12) = 1.77, H–C(13)); 7.15 (*d*, *J*(7,8) = 3.54, H–C(7)); 9.52 (*s*, H–C(15)).

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