194. X-Ray and ^{1}H-NMR Configurational Assignment of Valofan (α-Allophanoyl-α-allyl-γ-valerolactone) Diastereoisomers

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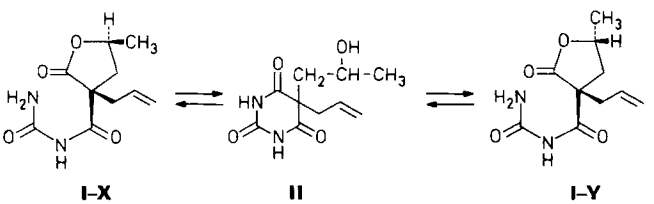
Summary

The two diastereoisomers of valofan were separated by semi-preparative HPLC. The preferred solution conformation of the two diastereoisomers was established by ^{1}H-NMR spectroscopy. The spectra could be interpreted in configurational terms. The configuration of the more lipophilic isomer was ascertained by X-ray crystallography as *r*-2-allophanoyl-2-allyl-*t*-4-methylbutyrolactone.

1. Introduction. – Valofan (**I**, α-allophanoyl-α-allyl-γ-valerolactone) is a drug marketed for the treatment of migraine. While its impact is clearly neurological, its mechanism of action remains essentially unknown. At high doses, it decreases the release of dopamine in rodent brains; this effect, however, is not associated with a direct action on dopamine receptors [1]. Valofan closely resembles α,γ-substituted γ-butyrolactones, a number of which display remarkable anticonvulsant properties, possibly by acting on a chloride channel regulated by γ-aminobutyric acid [2].

Valofan (**I**) exists in a relatively fast equilibrium with proxibarbal (**II**, 5-allyl-5-(2-hydroxypropyl)barbituric acid) *(Scheme 1)*. This interconversion occurs in aqueous solution as well as in biological systems [3] [4] and is of particular significance for the metabolism of valofan and proxibarbal in the human ([5] and ref. therein).

Scheme 1. *The Interconversion of Valofan Diastereoisomers* (**I X** and **I–Y**) via *Proxibarbal* (**II**). Only relative configurations are indicated.



I–X **II** **I–Y**

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Containing two centres of chirality, valofan exists as two diastereoisomers which are separable by HPLC. In [3], we designated as **X** the more lipophilic isomer which elutes first from silica columns, and as **Y** the more hydrophilic isomer which elutes first from reversed-phase columns. In the solid state and in aqueous solution, the **X**/**Y** ratio is very close to 1:2, the interconversion occurring in solution *via* proxibarbal *(Scheme 1)*. Prior to an extensive kinetic and thermodynamic study of these interconversion reactions (*Wittekind, Testa, Estreicher & Balant,* to be published), it became essential to establish the configuration of the **I–X** and **I–Y** diastereoisomers. The configurational assignment was achieved by ^{1}H-NMR spectroscopy and X-ray crystallography as reported here.

2. Experimental. – *Compounds.* Valofan (= N-*(aminocarbonyl)-5-methyl-2-oxo-3-(2-propenyl)tetrahydrofuran-3-carboxamide*) was generously donated by *Hommel AG* (Adliswil, Switzerland). The two diastereoisomers were separated as described in [3] by semi-prep. HPLC on a *Lichrosorb SI60* column with hexane/EtOH 96:4 as the mobile phase. The yield was 3 parts of **I X** for 4 parts of **I–Y**. NMR analysis showed **I–X** and **I–Y** to be contaminated with each other to a limited degree, < 10% and about 15%, respectively, for the samples studied by ^{1}H-NMR spectroscopy.

^{1}H-NMR Spectroscopy. The ^{1}H-NMR spectra were obtained at 360 MHz on a *Bruker WH-360* instrument operating under *Aspect 2000* control. A spectral width of 3.8 kHz was used with 32 K data points, resulting in an acquisition time of 4.3 sec and a digital resolution of 0.23 Hz/pt. Single-phase detection was employed with phase cycling. Solutions (0.01M) were made in $CDCl_3$ and measured at 24 ± 1°. The chemical shifts are in ppm relative to internal TMS. Theoretical spectra were calculated using the program *PANIC.*

Nuclear-*Overhauser*-effect difference experiments were run according to *Hall & Sanders* [6] by repetition of the sequence: preirradiation (5 sec), delay (2 msec), 90° pulse and acquisition of the FID. Difference spectra were generated by substracting the FID of each individual preirradiation from the control FID with the preirradiation frequency in off-resonance.

X-Ray Crystallography. A sample of **I–X** containing less than 5% of **I–Y** (HPLC analysis) was obtained. Crystals were grown from hexane/EtOH 95:5 at 5°. X-Ray intensity-data collection was carried out at 120°K with an *Enraf-Nonius CAD-4* automatic diffractometer.

3. Results. – 3.1. *^{1}H-NMR Analysis.* Well-resolved ^{1}H-NMR spectra of the diastereoisomers **I–X** and **I–Y** have been obtained in $CDCl_3$ at 24°C. Proton connectivities have been established by decoupling techniques. Spectral analysis including simulation gave the chemical shifts, and coupling constants reported in *Table 1.*

The spectra of the two diastereoisomers show a marked degree of similarity which should render difficult any direct configurational assignment. In particular, the vicinal couplings of proton H_a with the geminal protons H_c and H_d were comparable for both diastereoisomers (no individual assignment of H_c and H_d is possible from the analysis of the *ABX*-spin system). To assign the spectra to a specific configuration, it was therefore necessary to proceed indirectly, namely by first deducing conformational features from the spectra.

In **I–X**, as well as in **I–Y**, both vicinal couplings ^3J(a,c) and ^3J(a,d) are large (> 5.5 Hz) *(Table 1)*. In a three-spin system where the dihedral angle between the geminal protons is *ca.* 120°, this implies [7] that H_a must lie 'outside' the projection of the *gem*-protons H_c and H_d (**A**) and not 'inside' their projection (**B**) *(Scheme 2)*. The vicinal couplings are thus characteristic of axial interactions indicating that in both diastereoisomers the CH_3-group is in an equatorial position, and suggesting the 5-membered ring to adopt an envelope conformation with C(3) lying outside the plane of the four other ring atoms (for a definition of atoms see the *Figure*). The finding that the CH_3-group is equatorial in both compounds is confirmed by the similar chemical shift of the CH_3-resonance in the two isomers *(Table 1)*.

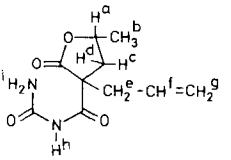


Table 1. *Chemical Shifts* (ppm relative to TMS) *and Coupling Constants* (Hz) *in the 360-MHz ^{1}H-NMR Spectrum of Valofan Diastereoisomers* (**I–X**: allophanoyl/CH_3 *trans;* **I–Y**: allophanoyl/CH_3 *cis*) *in $CDCl_3$ at 24°C*

Chemical shifts			Coupling constants		
Assignment	**I–X**	**I–Y**	Assignment	**I–X**	**I–Y**
H_b	1.453	1.426	^3J(a,b)	6.2	6.1
H_c	1.791	2.451[a])	^3J(a,c)	9.3	6.6
H_{e_1}	2.619	2.636	^3J(a,d)	6.4	9.0
H_{e_2}	2.705		^2J(c,d)	−13.5	−13.8
H_d	3.113	2.381[a])	$^2J(e_1,e_2)$	−14.1	[b])
H_a	4.560	4.634	$^3J(e_1,f)$	6.7	7.4
H_{g_1}	5.215	5.238	$\lvert^4J(e_1,g_1)\rvert$[c])	1.2	1.2
H_{g_2}	5.237	5.254	$\lvert^4J(e_1,g_2)\rvert$[c])	0.8	0.7
H_f	5.614	5.687	$^3J(e_2,f)$	7.8	7.4
H_{i_1}	5.41	5.71	$\lvert^4J(e_2,g_1)\rvert$[c])	1.0	1.2
H_{i_2}	7.88	7.90	$\lvert^4J(e_2,g_2)\rvert$[c])	0.6	0.6
H_h	8.50	9.25	$^3J(f,g_1)$[d])	17.0	16.6
			$^3J(f,g_2)$[d])	10.1	10.2
			$^2J(g_1,g_2)$	1.7	1.5

[a]) Assignment of the resonance at higher field to H_d is necessary because of the large coupling expected for ^3J(a,d) (see text).

[b]) The geminal coupling constant cannot be determined since H_{e_1} and H_{e_2} are chemically equivalent in **I–Y**.

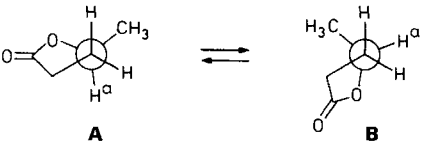
[c]) Sign of the allylic coupling constants is not reflected in the spectra.

[d]) Magnitude of the coupling constants assigns H_{g_1} as *trans* relative to H_f.

The chemical shifts of H_c and H_d are comparable in **I–Y**, but differ markedly in **I–X**. This means that the CH_2-protons in the two diastereoisomers experience differently the magnetic anisotropy shielding of the allophanoyl C=O group. A molecular model reveals that in **I–X** the allophanoyl moiety must be *trans* to the CH_3-group if it is to exert a strong deshielding on only one of the two geminal protons, namely H_d. This assigns proton H_d as equatorial, as confirmed by the value of the coupling constants, ^3J(a,d) = 6.4 Hz $\approx$ ^3J(ax,eq) and ^3J(a,c) = 9.3 Hz $\approx$ ^3J(ax,ax). These assignments are confirmed by nuclear-*Overhauser*-effect (NOE) difference spectroscopy: preirradiation of H_a led to a NOE enhancement (*ca.* 3%) of H_d but did not affect H_c. This corresponds to the shorter interproton distance between H_a,H_d (ax,eq) than between H_a,H_c (ax,ax).

In diastereoisomer **I–Y**, the allophanoyl moiety must be *cis* to the CH_3-group to allow for an equal distribution of its deshielding effect on H_c and H_d. Now H_d is

Scheme 2. *Two Possible Conformations of the Lactone Ring in Valofan.* Conformation **A** (equatorial CH_3-group) is the preferred one in both diastereoisomers.



assigned as axial and should have the larger coupling constant, $^3J(a,d) = 9.0$ Hz $\approx {}^3J(ax,ax)$, $^3J(a,c) = 6.6$ Hz $\approx {}^3J(ax,eq)$. Confirmation was obtained from the NOE (*ca.* 4%) observed for H_c when H_a was preirradiated.

Table 1 also suggests that the conformational behaviour of the allophanoyl moiety may be different in the two valofan diastereoisomers. The deshielding of H_h in **I–Y** is indicative of a H-bond between N(1) and O(2) (for a definition of atoms see the *Figure*). Molecular models indicate that this H-bond positions the allophanoyl moiety in such a manner that it exerts a similar deshielding on H_c and H_d, as indeed observed. We recall that such an intramolecular H-bond was postulated from lanthanide-induced shifts of **I** in CD_3CN solution [8], the **X**/**Y** ratio in I being close to 1:2 [3].

3.2. *X-Ray Analysis.* The crystal data, intensity collection, structure solution and refinement methods are summarised in *Table 2.* The measured intensities were corrected for absorption as before [9] and the variances of the intensities were derived from counting statistics and the fluctuations of three periodically measured check reflections.

Table 2. *Summary of Crystal Data, Intensity Measurements, Structure Solution and Refinement*

Formula	$C_{10}H_{14}N_2O_4$	μ [cm^{-1}]	1.16
Molecular weight	226.23	Scan method	2θ–θ
Crystal dimensions [mm]	0.25 × 0.19 × 0.06	$(\sin\theta/\lambda)_{max}$	0.60
Crystal system	Monoclinic	No of unique reflections	1929
a [Å]	7.183 (3)	No of reflections < 3σ	863
b [Å]	15.517 (3)	No of observations/	
c [Å]	10.366 (5)	No of variables	10.3 (8.3)[a]
β [°]	108.71 (4)	Structure solution	*MULTAN & Fourier*
U [Å^3]	1094.3	Refinement method	Full matrix least
Z	4		squares
$d_{calc.}$ [g · cm^{-3}]	1.37	Function minimized	$\Sigma w(\|F_o\| - \|F_c\|)^2$
F_{000}	480	w	$1/\sigma^2$
Space group	$P2_1/n$	R	0.053
Radiation	Mo$K\alpha$	R_w	0.044
λ [Å]	0.71069	Goodness of fit	1.36

[a]) Only those reflections with $I < 3\sigma$ with $|F_c| > |F_o|$ were included in the refinement.

The computer programs used for data reduction and structure solution were taken from the *XRAY-72* program system [10]. The scattering factors for the neutral non-H-atoms were taken from *Cromer & Mann* [11], and for H-atoms from *Stewart et al.* [12]. Starting phases were generated by the program *MULTAN* [13], the subsequent E-map revealing all the non-H-atoms. Refinement by full matrix least squares to $R = 0.093$, followed by a difference *Fourier* synthesis revealed all the H-atoms. Refinement was continued to $R = 0.053$. In the last cycles the non-H-atoms were refined anisotropically, and the H-atoms constrained to have the same isotropic temperature factor. The final atomic coordinates are reported in *Table 3.* Calculated bond lengths and angles are reported in *Table 4.* A perspective drawing of the molecule prepared by the program ORTEP [14] is shown in the *Figure.* The H-atoms are represented as spheres with an isotropic temperature factor artificially fixed at 0.004 Å^2 for clarity. Tables of the observed and calculated structure and temperature factors are available from the authors upon request.

The X-ray analysis unambiguously establishes that in the investigated compound the CH_3-group is *cis* to the allyl group and *trans* to the allophanoyl moiety. The chromatographically more lipophilic diastereoisomer of valofan can thus be designated as *r*-2-allophanoyl-2-allyl-*t*-4-methylbutyrolactone (**I–X**) according to IUPAC recommendations [15], while the other isomer is *r*-2-allophanoyl-2-allyl-*c*-4-methylbutyrolactone (**I–Y**).

Table 3. *Final Atomic Co-ordinates for* $C_{10}H_{14}N_2O_4$

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
C(1)	0.7504 (5)	0.0211 (2)	0.0635 (4)	O(4)	1.2347 (4)	−0.0039 (2)	0.5166 (2)
C(2)	0.7764 (5)	0.1126 (2)	0.1264 (4)	H(1)	0.949 (5)	0.046 (3)	0.360 (3)
C(3)	0.7442 (6)	0.1688 (3)	−0.0005 (4)	H(4)	0.952 (5)	0.115 (2)	−0.089 (3)
C(4)	0.8047 (6)	0.1124 (3)	−0.0989 (4)	H(7)	0.617 (5)	0.265 (2)	0.202 (3)
C(5)	0.6892 (8)	0.1249 (3)	−0.2456 (4)	H(21)	1.506 (5)	0.077 (2)	0.466 (3)
C(6)	0.6275 (6)	0.1294 (3)	0.2021 (4)	H(22)	1.343 (5)	0.132 (2)	0.350 (4)
C(7)	0.6516 (6)	0.2172 (3)	0.2647 (4)	H(31)	0.809 (5)	0.221 (2)	0.014 (3)
C(8)	0.7199 (6)	0.2320 (3)	0.3962 (4)	H(32)	0.597 (5)	0.183 (2)	−0.043 (3)
C(9)	0.9880 (5)	0.1181 (2)	0.2197 (4)	H(51)	0.536 (5)	0.111 (2)	−0.262 (3)
C(10)	1.2180 (5)	0.0517 (3)	0.4287 (3)	H(52)	0.692 (5)	0.185 (2)	−0.275 (3)
N(1)	1.0318 (4)	0.0693 (2)	0.3360 (3)	H(53)	0.719 (5)	0.093 (2)	−0.302 (3)
N(2)	1.3680 (5)	0.0971 (2)	0.4160 (3)	H(61)	0.494 (5)	0.123 (2)	0.135 (3)
O(1)	0.7673 (4)	0.0234 (2)	−0.0619 (2)	H(62)	0.645 (5)	0.081 (2)	0.270 (3)
O(2)	0.7190 (4)	−0.0443 (2)	0.1149 (3)	H(81)	0.740 (5)	0.287 (2)	0.432 (3)
O(3)	1.1105 (4)	0.1625 (2)	0.1922 (2)	H(82)	0.752 (5)	0.180 (2)	0.459 (3)

The lactone ring is in an envelope conformation as also deduced from the NMR spectrum. The atoms C(1), C(2), C(4) and O(1) lie in a plane which also contains O(2). The atom C(3) is located outside this plane with an elevation of 0.45 Å, and on the same side as C(5) and C(6). An additional conformational feature revealed by the X-ray analysis is the existence of an intramolecular H-bond between H(22) and O(3), with H(22)–O(3) = 1.98 Å and N(2)–H(22)–O(3) = 136°. Such a H-bond stabilizes a cyclic conformation of the allophanoyl moiety; it was previously postulated [8] from lanthanide-induced shifts in the ^{1}H-NMR spectrum of valofan (as a mixture of diastereoisomers) in CD_3CN. In contrast, an intramolecular H-bond between N(1) and O(2) does not exist in the crystalline state of the investigated valofan diastereoisomer (distance 2.91 Å).

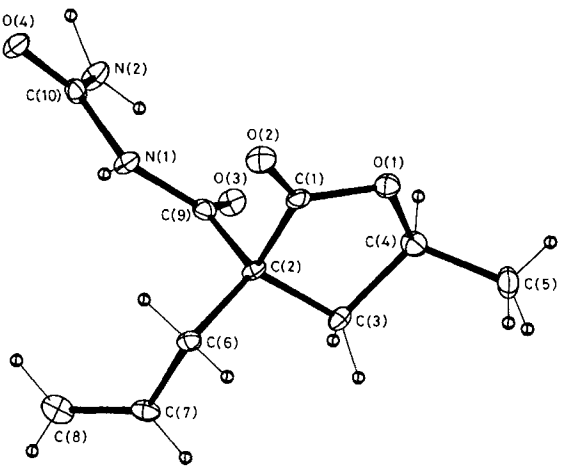


Figure. *The crystal structure of the more lipophilic valofan diastereoisomer* r-*2-allophanoyl-2-allyl-*t-*4-methyl-butyrolactone* (**I–X**)

Table 4. *Bond Lengths* (Å) *and Angles* (°) *for* $C_{10}H_{14}N_2O_4$ *with Estimated Standard Deviations in Parentheses*

a) Distances					
C(1)–O(1)	1.344 (5)	C(7)–C(8)	1.312 (6)	C(5)–H(52)	0.98 (4)
C(1)–O(2)	1.201 (5)	C(9)–O(3)	1.222 (5)	C(5)–H(53)	0.85 (4)
C(1)–C(2)	1.548 (5)	C(9)–N(1)	1.373 (5)	C(6)–H(61)	0.99 (3)
C(2)–C(3)	1.532 (5)	N(1)–C(10)	1.400 (4)	C(6)–H(62)	1.02 (3)
C(2)–C(6)	1.539 (6)	C(10)–O(4)	1.232 (5)	C(7)–H(7)	0.96 (3)
C(2)–C(9)	1.519 (5)	C(10)–N(2)	1.329 (5)	C(8)–H(81)	0.92 (4)
C(3)–C(4)	1.509 (6)	C(3)–H(31)	0.92 (4)	C(8)–H(82)	1.01 (3)
C(4)–C(5)	1.493 (5)	C(3)–H(32)	1.03 (3)	N(1)–H(1)	0.80 (4)
C(4)–O(1)	1.481 (5)	C(4)–H(4)	1.03 (4)	N(2)–H(21)	1.01 (3)
C(6)–C(7)	1.496 (6)	C(5)–H(51)	1.08 (4)	N(2)–H(22)	0.84 (4)
b) Angles					
O(1)–C(1)–O(2)	122.7 (3)	C(3)–C(4)–H(4)	115 (2)	C(7)–C(8)–H(81)	123 (2)
O(1)–C(1)–C(2)	110.3 (3)	C(5)–C(4)–O(1)	107.3 (3)	C(7)–C(8)–H(82)	118 (2)
O(2)–C(1)–C(2)	127.1 (4)	C(5)–C(4)–H(4)	108 (2)	H(81)–C(8)–H(82)	120 (3)
C(1)–C(2)–C(3)	101.3 (3)	O(1)–C(4)–H(4)	106 (2)	C(2)–C(9)–O(3)	122.1 (3)
C(1)–C(2)–C(9)	106.3 (3)	C(4)–C(5)–H(51)	110 (2)	C(2)–C(9)–N(1)	115.4 (3)
C(1)–C(2)–C(6)	111.1 (3)	C(4)–C(5)–H(52)	112 (2)	O(3)–C(9)–N(1)	122.5 (3)
C(3)–C(2)–C(6)	113.8 (3)	C(4)–C(5)–H(53)	117 (2)	N(1)–C(10)–N(2)	117.1 (3)
C(3)–C(2)–C(9)	111.1 (3)	H(51)–C(5)–H(52)	105 (3)	N(1)–C(10)–O(4)	119.2 (3)
C(6)–C(2)–C(9)	112.5 (3)	H(51)–C(5)–H(53)	104 (3)	N(2)–C(10)–O(4)	123.7 (3)
C(2)–C(3)–C(4)	104.7 (3)	H(52)–C(5)–H(53)	108 (3)	C(1)–O(1)–C(4)	110.8 (3)
C(2)–C(3)–H(31)	115 (2)	C(2)–C(6)–C(7)	111.9 (3)	C(9)–N(1)–C(10)	127.5 (3)
C(2)–C(3)–H(32)	110 (2)	C(2)–C(6)–H(61)	107 (2)	C(9)–N(1)–H(1)	122 (2)
C(4)–C(3)–H(31)	112 (3)	C(2)–C(6)–H(62)	106 (2)	C(10)–N(1)–H(1)	110 (2)
C(4)–C(3)–H(32)	108 (2)	H(61)–C(6)–H(62)	107 (3)	C(10)–N(2)–H(21)	119 (2)
H(31)–C(3)–H(32)	107 (3)	C(6)–C(7)–C(8)	124.3 (4)	C(10)–N(2)–H(22)	117 (3)
C(3)–C(4)–C(5)	115.3 (4)	C(6)–C(7)–H(7)	116 (2)	H(21)–N(2)–H(22)	122 (3)
C(3)–C(4)–O(1)	104.5 (3)	C(8)–C(7)–H(7)	120 (2)		

4. Discussion. – The configuration established by X-ray crystallography confirms the ^{1}H-NMR deduction and indicates the correctness of the reasoning. However, and as pointed out earlier, a direct configurational assignment based on ^{1}H-NMR spectra is not straightforward. Compare for example the spectra reported in *Table 1* with those measured by *Altman et al.* [16] for compounds analogous to valofan, but lacking a 2-allyl substituent. In the latter compounds, the *gem*-protons on C(3) have similar chemical shifts in the *trans*-diastereoisomers, and markedly different ones in the *cis*-isomers, in contrast to the behaviour of valofan diastereoisomers. This discrepancy originates from a different conformational behaviour of the lactone ring in the two systems (2,2,4-trisubstituted *vs.* 2,4-disubstituted), and indeed proves that in such compounds reliable configurational assignments must be preceded by unambiguous conformational deductions. The danger of circular reasoning must be borne in mind.

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