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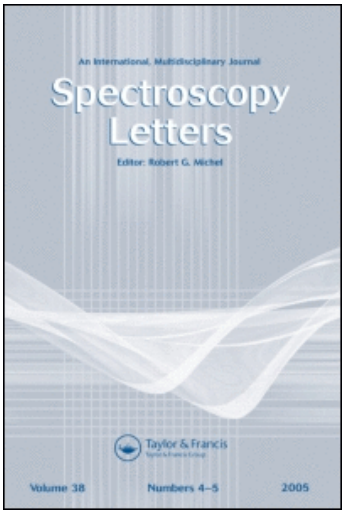

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^{13}C NMR Study of the Natural Glycosides Vicine and Convicine

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^{13}C NMR STUDY OF THE NATURAL GLYCOSIDES VICINE AND CONVICINE

Keywords: ^{13}C NMR, glycosides, natural products

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ABSTRACT

^{13}C NMR parameters have been obtained for vicine and convicine in DMSO, D_2O/DMSO and D_2O. Complete assignment of the spectra has been achieved. Interpretation of spin-lattice relaxation rates and heteronu-

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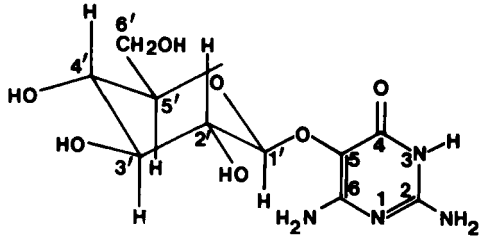
clear NOEs has yielded evidence of intramolecular structuring in the case of vicine and not in that of convicine and also of a complex network of solute-solvent interactions.

INTRODUCTION

Vicine (I) and Convicine (II) (figure 1) are glycosides isolated from Vicia faba. Their aglycones have been shown to be active principles, responsible for haemolitic crysis in sensitive individuals.

These substances were isolated more than 90 years ago, and different structures have been suggested.[1,2] Structures (I) and (II) have been proposed on the basis of synthesis of both compounds,[3] supported by ^{1}H NMR spectroscopy,[4] spectrophotometric data[5] and enzymatic methods.[6] Although pathological effects of these glycosides have been extensively investigated, comprehensive structural studies have not been carried out



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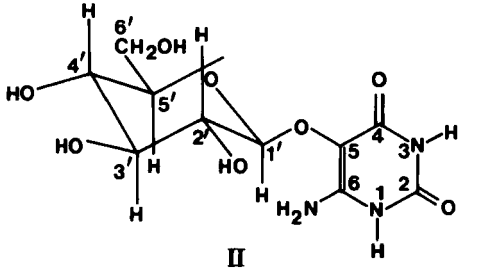

II

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so far; ^{1}H NMR spectra have been reported, yielding only limited information on the glycosidic ring protons.[4]

These considerations led us to investigate ^{13}C NMR parameters of vicine and convicine in order i) to get univocal characterization of both glycosides, ii) to investigate the motional behaviour of these molecules by ^{13}C NMR dynamic parameters (T_1 and NOE) and iii) to achieve information about physicochemical properties depending on the solvent system.

This preliminary study will be extended to characterize, in vitro, the behaviour and reactivity of vicine and convicine in erythrocytes of sensitive individuals.

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EXPERIMENTAL

Both glycosides were obtained from Vicia faba according to the method of extraction by Bien et al.[4] ^{13}C NMR spectra were obtained at 301±1 K on a Bruker WH-90 FT-NMR spectrometer operating at 22.63 MHz. The concentrations were 100 mg/ml in DMSO-d_6 or in D_2O or in DMSO-d_6/D_2O 1:1. The pH of aqueous solutions was adjusted with either DCl or NaOD.

Spin-lattice relaxation rates were measured by using inversion recovery pulse sequences $(180°-\tau\ 90°-t)_n$ and $^{13}C-\{^{1}H\}$ NOEs were measured with gated decoupling techniques. $1/T_1$ values were calculated by a three parameter regression analysis of the recovery curves of longitudinal magnetization components.

RESULTS AND DISCUSSION

The study has been carried out primarily in DMSO-d_6 due to the high solubility of vicine and convicine; moreover DMSO has been shown to provide the most suitable solvent for studying anomerism of sugars and tautomerism of pyrimidines.[7,8] Spectra were also recorded in a mixture D_2O/DMSO 1:1. In pure water, vicine is slightly soluble and convicine

gets soluble only at high temperatures; both substances however are rather soluble in dilute alkalies and acids; further information about the assignment of ^{13}C NMR spectra can be therefore obtained by observing the changes in chemical shifts as a function of pH, that is to say by monitoring the protonation and deprotonation processes.

Assignments were made on the basis of a) off resonance decoupling experiments, b) single resonance decoupling experiments, c) comparison with model compounds, d) solvent dependence of ^{13}C chemical shifts, e) pH dependence of ^{13}C spectra and f) spin-lattice relaxation rates and NOE's of individual carbons.

^{13}C chemical shifts of (I) and (II) and related assignments are reported in Table I for the different solvent systems. In DMSO-d_6 and D_2O glucopyranose ring carbons of vicine and convicine display the same values of chemical shifts.

By comparison with the chemical shift data of α and ß anomers of glucose,[9] it can be inferred that the pyranose ring of both glycosides is in the ß anomeric configuration. ^{1}H NMR spectra (not shown) confirm these findings: in fact, a vicinal coupling constant of 4.5 Hz, typical for the pyranose ring in the ß configuration[10] is measured for the H_1 doublet at 3.85 ppm. The shift itself of H_1 agrees with the occurrence of the ß configuration since the chemical shift of the α anomeric proton is around 5.25 ppm.[11]

Although glycosides and nucleosides have been extensively studied, univocal assignment of resonances within pyrimidine moieties of vicine and convicine is not trivial. A complete assignment cannot be achieved on the basis of comparison with model compounds nor it can be obtained by calculating substituent contributions to the chemical shifts: several peaks are crowding a small region of chemical shifts, thus making it hard to distinguish among different carbons. Moreover theoretical correlations of ^{13}C chemical shifts in similar systems have been demonstrated to yield ambiguous results.[12] Especially in the case of

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convicine, univocal assignment of C_2 and C_6 is not possible by considering only the chemical shifts. The peak at 110.52 ppm in DMSO-d_6 can be attributed to C_5 by comparison with the spectra of uridine and of 5-hydroxy-uridine and by considering the deshielding effect of the $-NH_2$ group on C_6. The most deshielded resonance has been assigned to C_4 by comparison with ^{13}C spectra of model compounds but some ambiguity was still remaining on the assignment of C_2 and C_6 (149.18 and 150.41 ppm respectively). Spectra at different pH in water (see Table I) provided a suitable aid, since C_6 is expected to be much more affected than C_2 by protonation equilibria. Thus a different spectral behaviour in acid and alkaline solutions is expected. The extreme pH values used (pH = 10.0 or 4.0) have been choosen in order to avoid hydrolysis of the glycosidic linkage.

^{13}C NMR spectra of vicine and convicine in DMSO-d_6/D_2O, as compared with those obtained in DMSO-d_6, only show the same overall change in chemical shifts of all resonances due to the different solvent system. In alkaline solution (pD = 10.0) resonances of convicine show a large downfield shift (~10 ppm), that clearly arises from the deprotonation process. The large downfield shift of C_4 in anionic form indicates that the keto-enolic tautomerization largely favours the enolic form. On the other hand, in acid solution (pD = 4.0), the resonance at 150.51 ppm moves 2 ppm upfield, while that at 151.18 is not affected; the first one can be therefore assigned to C_6.

The most significant pH-dependent changes in ^{13}C chemical shifts of vicine are measured for the resonances at 158.69 and 151.61 ppm in DMSO-d_6. On the basis of such pH dependence these resonances were assigned to C_6 and C_2; univocal assignment was then obtained on the basis of comparison with isocytosine.

^{13}C NMR spin-lattice relaxation rates and $^{13}C-\{^1H\}$ NOEs are reported in Tables II and III for vicine and convicine respectively. The NOEs of protonated glucopyranose carbons are very close to the maximum

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Table I

^{13}C NMR chemical shifts and assignments for vicine and convicine (100 mg/ml in every solvent) at 301 K

Substance	Peak	Solvent: DMSO-d_6	DMSO-d_6/D_2O 1:1	D_2O pD = 10
VICINE	C_2	158.69	160.45	169.66
	C_4	158.24	159.79	160.38
	C_5	113.29	114.94	117.96
	C_6	151.61	153.16	158.17
	$C_{1'}$	107.87	107.87	107.57
	$C_{2'}$	73.18	74.29	73.77
	$C_{3'}$	77.60	76.84	77.97
	$C_{4'}$	69.86	70.93	70.67
	$C_{5'}$	76.18	76.84	77.08
	$C_{6'}$	61.16	61.91	61.62
CONVICINE	C_2	150.29	151.18	161.48
	C_4	161.34	162.45	163.45
	C_5	110.52	111.63	114.20
	C_6	149.18	150.51	160.16
	$C_{1'}$	107.47	107.65	106.69
	$C_{2'}$	73.18	73.85	73.99
	$C_{3'}$	77.69	78.04	77.52
	$C_{4'}$	69.86	70.31	70.23
	$C_{5'}$	76.18	76.72	76.64
	$C_{6'}$	61.16	61.69	61.34

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Table II

22.63 MHz ^{13}C NMR spin-lattice relaxation rates and $^{13}C-\{^1H\}$ NOEs for vicine 100 mg/ml in DMSO-d_6 at 301 K

Peak	$(R_1)_{exp}$ (s^{-1})	NOE	Adiacent nitrogens	(R_1^{CH}) (s^{-1})	$(R_1^{CH})_{DD}$ (s^{-1})	Comments
$C_{1'}$	2.86	1.98				
$C_{2'}$	3.12	2.01				
$C_{3'}$	3.03	1.90				
$C_{4'}$	2.78	1.90				
$C_{5'}$	3.22	1.92				
$C_{6'}$	6.25	1.95				
C_2	0.21	1.52	3 at 1.3 Å	0.20	0.15	4 H at 2.2 Å
C_5	0.11	1.52	2 at 2.1 Å	0.11	0.08	1 H at 2.3 Å
C_6	0.23	0.69	1 at 1.3 Å	0.22	0.09	1 H at 1.9 Å 2 H at 2.2 Å 4 H at 2.4 Å
C_4	0.21	1.39	1 at 1.3 Å	(0.20)	(0.14)	1 H at 2.1 Å

value, thus demonstrating that the main relaxation mechanism is the $^{13}C-^1H$ dipolar interaction. From the data in tables II and III it is apparent that all protonated carbons exhibit the same normalized relaxation rate R_1/n_H (where n_H is the number of attached protons). As a consequence, motional features can be suitably approximated in terms of isotropic reorientational diffusion. The correlation times for such motion can be calculated at 0.13 ± 0.02 ns and 0.17 ± 0.03 ns for vicine and

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Table III

22.63 MHz ^{13}C NMR spin-lattice relaxation rates and $^{13}C-\{^{1}H\}$ NOEs for convicine 100 mg/ml in DMSO-d_6 at 301 K

Peak	$(R_1)_{exp}$ (s^{-1})	NOE	Adiacent nitrogens	(R_1^{CH}) (s^{-1})	$(R_1^{CH})_{DD}$ (s^{-1})	Comments
$C_{1'}$	3.70	1.80				
$C_{2'}$	4.35	1.70				
$C_{3'}$	3.70	1.80				
$C_{4'}$	4.35	1.80				
$C_{5'}$	3.85	1.90				
$C_{6'}$	6.25	1.80				
C_5	0.16	1.70	1 at 2.1 Å	0.16	0.14	1 H at 2.0 Å
C_6	0.32	1.00	2 at 1.3 Å	0.28	0.14	2 H at 2.1 Å
C_2	0.66	1.40	2 at 1.3 Å	(0.62)	(0.44)	1 H at 1.8 Å 2 H at 2.1 Å
C_4	0.22	2.00	1 at 1.3 Å	(0.20)	(0.20)	2 H at 2.2 Å

convicine respectively by using the formula of Allerhand et al. ($R_1/n_H = 2.0235 \times 10^{10} \tau_c$).[14]

As far as the quaternary carbons of the pyrimidine ring are concerned different contributions to the relaxation mechanism can be figured out, i.e. the dipolar interaction with ^{14}N, the dipolar interaction with ^{1}H and finally mechanisms other than the dipolar:

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$$(R_1)_{exp} = (R_1^{CN})_{DD} + (R_1^{CH})_{DD} + (R_1)_{other}$$

The $^{13}C-^{14}N$ dipolar relaxation term can be evaluated for an isotropic tumbling motion by the following equation:

$$(R_1^{CN})_{DD} = \frac{2}{15}\hbar^2 \gamma_C^2 \sum_{j=1}^{j=n} \gamma_j^2 \frac{1}{r_{Cj}^6} f(\omega_C, \omega_j, \tau_c)$$

These contributions can be substracted from the experimental relaxation rate yielding:

$$(R_1^{CH}) = (R_1)_{exp} - (R_1^{CN}) = (R_1^{CH})_{DD} + (R_1)_{other}$$

The values of R_1^{CH} thus obtained are also reported in Tables II and III. Furthermore, the $(R_1^{CH})_{DD}$ values can be obtained from the NOE data by using the well known equation

$$(R_1^{CH})_{DD} = (R_1^{CH})(1.98/NOE)$$

For quaternary carbons $(R_1^{CH})_{DD}$ were analyzed as generated by n neighbouring protons at a given r_{CH} distance, as commented in Tables II and III.

The same calculations were also attempted for carbonyl carbons, even though, strictly speaking, the approach is not very likely to be entirely correct since the ^{13}C relaxation of carbonyl carbons does not follow the same pathway as protonated and quaternary carbons. Nevertheless, the high NOE values measured in an aprotic solvent strongly suggest the possibility that carbonyls occur in a partially protonated form. Also these results are reported (in parenthesis) in Tables II and III. For vicine the comments reported in Table II for C_2 and C_6 agree with the features of the molecular structure; C_5 requires

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another proton at a distance of 2.3 Å. This condition cannot be satisfied by the amino protons attached to C_6 that are at a distance of about 2.6 Å. So it is possible that the second proton is the H_2 proton of th glucopyranose ring. This being the case, molecular models show that the pyrimidine ring should be close enough to the glucopyranose ring as to align the C_5-C_6 bond to the C_1-C_2 bond.

For convicine the comment reported in Table III for C_6 agrees with the closeness of the two amino protons; C_5 requires only one proton at 2.0 A; that may be one of the amino protons attached to C_6. In this case no proximity between the pyrimidine and the glucopyranose rings can be predicted.

Calculations for the carbonyls carbons (in parenthesis) yield a very uncommon number of protons that cannot be interpreted by the molecular formula for both vicine and convicine, thus suggesting that these groups experience a complex network of solute-solvent interactions.

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