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N-Salicylideneamino acidato complexes of oxovanadium(IV). The cysteine and penicillamine complexes†

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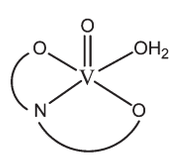
Oxovanadium(IV) complexes with ligands derived from the reaction of salicylaldehyde with L-cysteine and with D- and D,L-penicillamine are prepared. The compounds are characterised by elemental analysis, spectroscopy (UV-VIS, CD, EPR), TG, DSC and magnetic susceptibility measurements (9–295 K). We discuss several aspects related to the structure of these complexes in the solid state and in solution; in particular, the possibility of forming thiazolidine complexes, and their comparison with the characterised complexes is studied by molecular mechanics and density functional theory calculations. The solution structures depend on pH and solvent, and while with L-Cys the spectroscopic results show trends similar to those of the L-Ala and L-Ser systems up to *ca.* pH 8–9, where thiolate coordination starts being detected, the penicillamine system is quite distinct, namely thiolate coordination occurs for pH > 6.5. In the presence of salicylaldehyde and $V^{IV}O$ the desulfydration of cysteine proceeds rapidly, but no similar reaction occurs with penicillamine, although its decomposition is also activated. The DFT calculations do not indicate any energetic basis for this distinct reactivity, which possibly results from different complexes present in the Cys and Pen systems. In the cysteine system, the *N*-salicylidene dehydroalanine–$V^{IV}O$ complex **V** is believed to form in an intermediate stage of the desulfydration. Further, addition of several nucleophiles to the cysteine reaction mixtures produce amino acid derivatives by a Michael-type base-catalysed addition, a result compatible with the formation of **V**. The products of these reactions were analysed by TLC and HPLC, and in some cases isolated.

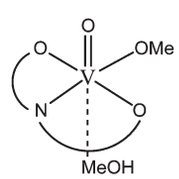
Introduction

Complexes of *N*-salicylideneamino acids have been the subject of extensive research, typified by their vanadium compounds.[1–16] With vanadium-(IV) and -(V) these Schiff base (SB) complexes often have coordination geometries such as those in **I** or **II**. In a few cases, dinuclear oxo-bridged V^{IV}–O–V^{V} or V^{V}–O–V^{V} compounds **III** have been obtained[4,5,11,12,16,17] and were characterised by X-ray diffraction.

Upon standing in methanolic solution in air $[V^{IV}O(sal–aa)(H_2O)]$ complexes (sal–aa = *N*-salicylidene-amino acidato) are spontaneously oxidised to $[V^{V}O(sal–aa)(OMe)(OHMe)]$ **II**. If 2,2′-bipyridine (bpy) or pyridine (py) is present in solution, they coordinate to $V^{IV}O^{2+}$, forming complexes with binding modes such as **IV**, where N∩N denotes bpy or 2 × py, and oxidation may be slowed down or avoided.[1,7,8,13,18]



I



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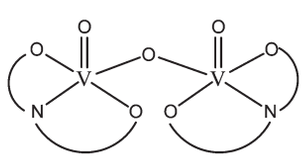
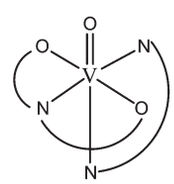


III



IV

In this work we isolate several oxovanadium(IV) complexes with ligands derived from the reaction of salicylaldehyde with L-cysteine and D- or D,L-penicillamine (hereafter designated by Pen), and we study several aspects related to the structure of these complexes in the solid state and in solution, including the use of molecular mechanics and density functional calculations.

The change in ligand reactivity caused by the coordination of amino acids to a wide variety of metal ions is well known. The activating effect of the metal ion may be extended by coordinating

† Electronic supplementary information (ESI) available: ESI-1 Some TLC data for the $V^{IV}O$/HSal/aa mixtures (aa = Cys, Pen, CysMe, CysEt) and the corresponding complexes in ethanol. ESI-2 Preparation of complexes **2–9**. ESI-3 Racemization experiments. ESI-4 Synthesis of amino acids based on the dehydroalanine complex **V**. ESI-5 Differential scanning calorimetric curves. ESI-6 Circular dichroism spectra. ESI-7 CD and EPR spectra of aqueous–ethanolic (3 : 1) solutions containing amino acid : salicylaldehyde : $V^{IV}O$ (1 : 1 : 1). ESI-8 Chiral-HPLC. ESI-9 General information about the DFT-calculated structures. ESI-10 Molecular mechanics calculations. ESI-11 Isomers of the thiazolidine complexes $[V^{IV}O(tz–Cys)(H_2O)]$ and $[V^{IV}O(tz–Pen)(H_2O)]$. See http://www.rsc.org/suppdata/dt/b4/b404305g/

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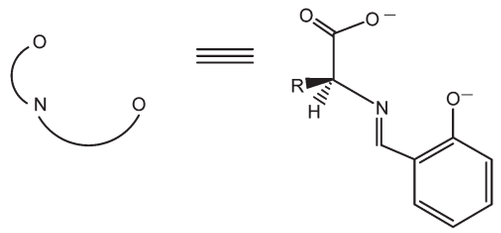
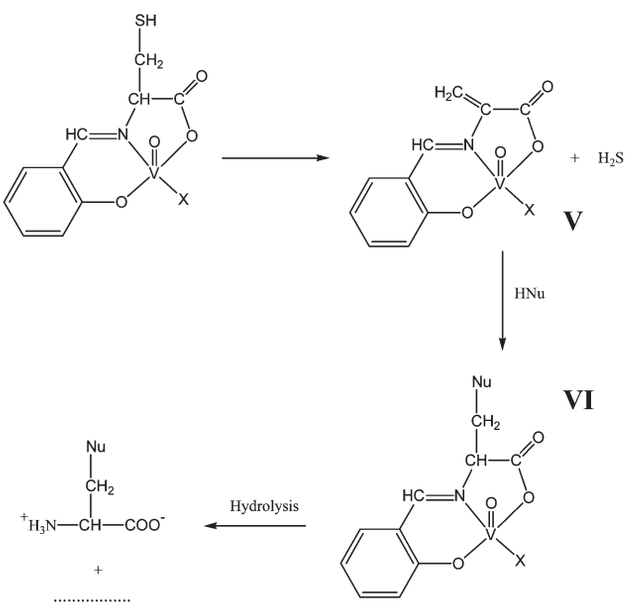


the amino acid as a Schiff base using a suitable carbonyl fragment such as pyridoxal, salicylaldehyde or pyruvate.[19,20] Snell[21,22] showed that many reactions involved in the metabolism of amino acids, *e.g.* transamination, α-decarboxylation, racemization, desulfydration *etc.*, catalysed by enzymes that require pyridoxal phosphate as a co-factor, could be simulated using pyridoxal, a metal ion (Fe^{3+}, Cu^{2+}, Al^{3+}) and the amino acid. Vanadium is one of the most active metal ions in β-eliminations, a process involved in *e.g.* desulfydration.[23–28] Bergel also showed that the desulfydration of cysteine is activated in the presence of pyridoxal and vanadium salts,[23,24] and this seems to provide a good analogy to the action of *cysteine desulfydrase*, a pyridoxal phosphate dependent enzyme.[29] However, no similar reaction occurred with penicillamine, although this also degrades. The decomposition of the amino acids activated by vanadium could explain the difficulty in isolating vanadium complexes involving Cys and Pen.

The desulfydration of cysteine also occurs using salicylaldehyde instead of pyridoxal. The *N*-salicylidenedehydroalanine complex **V** is believed to form in an intermediate stage (Scheme 1),[24] the decomposition of the amino acid leading to the formation of NH_3, pyruvic acid and other products. In this work we also show that addition of several nucleophiles (HNu) to the reaction mixture containing vanadium salts, salicylaldehyde (Hsal) and Cys produce amino acid derivatives by a Michael-type base-catalysed addition, a result compatible with the formation of **V** (sal–DHAla) and **VI**. The products of these reactions were analysed by means of TLC and HPLC, and in some cases isolated.



Scheme 1 Summary of the present reactions activated by oxovanadium(IV). HNu represents a nucleophile which may add to the β-carbon atom of the coordinated amino acid, and the dehydroalanine ligand in **V** will be designated by sal–DHAla.

Experimental

L-cysteine (Sigma), D-penicillamine (Sigma), salicylaldehyde (Aldrich), $VOSO_4{\cdot}5H_2O$ (Merck) or $VOSO_4{\cdot}H_2O$ (BDH), 2,2′-bipyridine (Merck), sodium acetate (Merck) lanthionine (BDH), *S*-methyl-L-cysteine (Sigma), *S*-ethyl-L-cysteine (Sigma), *S*-benzyl-L-cysteine (Aldrich), 2-mercaptoethanol (Merck), methanethiol (Aldrich), ethanethiol (Janssen), thiophenol (Merck), benzylmercaptan (Aldrich), dimethylamine (Carlo Erba), and solvents were used as purchased. *S*-hydroxyethylcysteine (see ESI-4†) and 2-amino-3-(diethylamino)-propanoic acid[30] were prepared for use as standards (for TLC and HPLC). Oxygen was removed from the solvents used for preparation or spectroscopic studies by bubbling N_2.

TLC experiments

Most preparations and syntheses were monitored by TLC, on Merck TLC plates (Art. 5626, 10 × 20 cm or Art. 5721, 20 × 20 cm). Samples of 1–5 μl of the reaction mixture were applied to the plates 20 mm from the bottom. In the experiments for synthesis of Cys derivatives **12**–**19**, normally two samples of the reaction mixtures were applied: (i) sample acidified with a strong acid (1–3 M HCl, H_2SO_4 or HNO_3), (ii) sample taken from the reaction flask and diluted (or not) with the same volume of water as of acid in (i). Elutions were carried out in Camag twin chambers with walls covered with filter paper impregnated with the eluent. Eluents used were: (**A**) ethanol/water (7 : 3), (**B**) butanol/ethanol/propionic acid/water (10 : 10 : 2 : 5), (**C**) chloroform/methanol/ammonia (17%) (2 : 2 : 1), (**D**) butanol/acetone/water/diethylamine (10 : 10 : 5 : 2). When the eluents reached approximately 120 mm from the bottom, the plates were removed and dried. The chromatogram was developed: (i) with ninhydrin–collidine–copper solution prepared according to Moffat and Lytle[31] and (ii) with iodine vapours in an enclosed chamber. In some cases the detection of sulfur-containing compounds used a spray of a $PdCl_2$/HCl solution. The presence of particular products in samples was confirmed by comparing their R_F values with standards and by spiking the samples with the corresponding standard, using the same TLC plate.

TLC experiments to monitor the preparation of complexes 2–9. All preparations were monitored by TLC and ESI-1† summarises the results. Distinct brown spots (Sp) corresponding to the vanadium complexes were always detected. Distinct spots (AA) were also detected at the R_F of the free amino acids and the brown spots Sp each tailed to the spot AA. This tailing was more severe for eluent **B** (acid eluent). Before development, depending on the sample, a pale blue spot (from $VOSO_4$) could frequently be detected at $R_F \approx 0$. In many cases, a weak bluish white continuous tailing could be seen up to the R_F of the complexes. All these TLC results are similar to those obtained in the preparations of [V^{IV}O(sal-L-Ala)(X)][7] and [V^{IV}O(dsal-L-aa)(X)] (X = H_2O, py or bpy; dsal = Hsal and derivatives).[13] The [V^{IV}O(sal-L-aa)(X)] complexes were eluted in the TLC plates presumably with X = EtOH, butanol, bpy or H_2O, the R_F values also being determined by the relative amount of EtOH in the eluent. As complexes containing bpy systematically presented lower R_F values, in spite of the fact that EtOH and H_2O are present in much higher concentrations, we conclude that the bpy–V bonds are the last to be hydrolysed during elution. In the very first stages of preparation of **2**–**7** (above), before the addition of the $VOSO_4$ solution (and before precipitation of the thiazolidines), three other weak spots were normally detected besides the cysteine or penicillamine spots: one corresponding to the aa disulfide, and two reddish/orange spots at R_F values similar to those of **2**–**7**. These two spots possibly correspond to the free Schiff bases and the thiazolidines.

TLC experiments to monitor the desulfydration of sulfur-containing amino acids and the preparation of compounds 12–19

All reactions were monitored by TLC and eluents **B**, **C** or **D** were used (always at least two eluents). In some cases 20 × 20 cm plates were used and were divided in two parts, 10 × 20 cm each, an equal set of samples being applied on each part. After elution and drying the plates, one half was covered with a 10 × 20 cm glass, and the other half developed with the ninhydrin–collidine–copper, followed, after *ca.* 2–5 h, by iodine vapours. The second half was developed later with a spray of the $PdCl_2$/HCl solution, to detect the S containing compounds.

Table 1 Elemental analysis of VO(sal–aa)(X) compounds (aa = sulfur containing amino acids; X = H_2O or bpy)[a]

Compound	%C	%H	%N	%S	%V	Colour
2 [VO(sal–L-Cys)(H_2O)]	39.2	3.6	4.5	10.5	16.6	Greyish-blue
Calculated: for $C_{10}H_{11}NSO_5V$	38.97	3.60	4.54	10.40	16.53	
3 [VO(sal–L-Cys)(bpy)]·1·2H_2O	51.3	3.9	8.7	6.6	n.a.	Orange
Calculated for: $C_{20}H_{19.4}N_3SO_{5.2}V$	51.33	4.18	8.98	6.85		
4 [VO(sal–D-Pen)(H_2O)]	44.7	4.7	4.0	9.7	15.3	Green
Calculated for: $C_{12}H_{15}NSO_5V$[b,c]	45.00	4.72	4.37	10.01	15.9	
5 [VO(sal–D,L-Pen)]·0.2H_2O·0.8EtOH	45.8	5.0	3.9	9.3	13.6	Orange
Calculated for: $C_{13.6}H_{18.2}NSO_5V$[b]	45.54	4.67	3.90	8.94	14.2	
6 [VO(sal–D-Pen)(bpy)]·0.8H_2O·0.8EtOH	53.6	4.7	7.8	6.1	9.5	Orange
Calculated for: $C_{23.6}H_{27.4}N_3SO_{5.6}V$[c]	53.92	4.95	7.99	6.10	9.69	
7 [VO(sal–D,L-Pen)(bpy)]·1.0H_2O	53.6	4.7	7.8	6.1	9.6	Orange
Calculated for: $C_{22}H_{23}N_3SO_5V$	53.92	4.95	7.99	6.10	9.69	
8 [VO(sal–CysSMe)(bpy)]·0.8EtOH	54.0	4.9	8.2	6.6	n.a.	Orange
Calculated for: $C_{22.6}H_{22.2}N_3SO_{4.8}V$	53.66	4.71	8.53	6.51		
9 [VO(sal–CysSEt)(bpy)]·1.2H_2O·0.3EtOH	53.0	4.9	8.1	6.1	10.0	Orange
Calculated for: $C_{22.8}H_{25.8}N_3SO_{5.6}V$	53.23	4.90	8.17	6.23	9.90	
10 (tz–L-Cys)·0.15H_2O	52.5	4.9	6.0	14.3		White
Calculated for: $C_{10}H_{11}NSO_3$·0.15H_2O	52.69	5.00	6.14	14.07		
11 (tz–D-Pen)·0.7H_2O	54.3	6.0	5.3	12.3		White
Calculated for: $C_{12}H_{15}NS\ O_3$·0.8H_2O	54.20	6.22	5.27	12.06		

[a] Compound **1** is brown and its formulation, as determined by X-ray diffraction,[7] corresponds to [VO(sal–L-Ala)(bpy)]·1/8H_2O·1/2EtOH. A few other compounds of the type [VO(sal–L-aa)(bpy)] (aa = several) are known[13] and their colour is brick-red. [b] The analytical results give better fits with formulations including 0.1–0.2 further moles of H_2O per V atom. [c] The CD signals of **4** and **6** were very weak, indicating that the amino acid partly racemized.

HPLC experiments

These used a Jasco HPLC system including Jasco 870-UV (absorbance) and 821-FP (fluorescence) detectors. A Rheodyne 7125 injection valve (20 µl loop) and a reverse phase column (LiChrosorb C_{18}-5 µm particles, 250 × 4 mm) were used. Eluents depended on the samples to be analysed (see below); they were always filtered before use (45 µm filters) and degassed; its flow rate was 1.0 ml min^{-1}.

Amino acids were analysed using three different precolumn derivatizations: (**A**) the usual *o*-phthaldialdehyde/mercaptoethanol procedure for amino acid analysis,[32] or (**B**) the iodoacetate/*o*-phthaldialdehyde/mercaptoethanol[33] procedure for cysteine and penicillamine and, (**C**) the *o*-phthaldialdehyde/chiral mercaptans procedure[34] to separate optically active amino acids: *N*-acetyl-L-cysteine (NAC) and *N*-acetyl-D-penicillamine (NAP) were used for this purpose. The presence of products in samples was confirmed by comparing their retention times with standards, and by spiking the samples with the corresponding standard.

Preparation of complexes 2–9

Complexes [VO(sal-L-Cys)(H_2O)] **2**, [VO(sal-L-Cys)(bpy)]·*n*H_2O **3**, [VO(sal-D-Pen)]·*n*H_2O·*m*EtOH **4**, VO(sal-D,L-Pen)·*n*H_2O·*m*EtOH **5**, [VO(sal-D-Pen)(bpy)]·*n*H_2O·*m*EtOH **6**, [VO(sal-D,L-Pen)-(bpy)]·*n*H_2O **7**, [VO(sal-CysSMe)(bpy)]·*m*EtOH **8**, [VO(sal-CysSEt)(bpy)]·*m*H_2O **9** (CysSMe = *S*-methyl-L-cysteine and CysSEt = *S*-ethyl-L-cysteine) were obtained by the general procedure described below. The preparations were carried out with deoxygenated solutions and under N_2.

General procedure. The amino acid was dissolved in an aqueous solution of sodium acetate and a solution of salicylaldehyde in ethanol added. The solution became yellow and shortly afterwards the white thiazolidine precipitated, except in the cases of **8** and **9**. An aqueous solution of vanadyl sulfate was added, and most of the white solid dissolved. In the case of **3** and **6**–**9** solid bpy was added either before (**3**) or after (**6**–**9**) the addition of $VOSO_4$. The mixture was filtered within 5–10 min and the remaining white solid separated. After 20–30 min, the complexes precipitated, and were separated by filtration and washed. Table 1 summarizes the analytical results, ESI-1† the monitoring of the preparations by TLC and ESI-2† the details of the preparations.

Desulfydration mixtures

Cysteine. The solutions used to study the desulfydrations contained equimolar amounts of L-Cys, $VOSO_4$·5H_2O and Hsal. Experiments were carried out with stirring at 40 °C and at the pH values: 3, 5.3, 6, 8, 9, in 250 ml 3/4 necked round flasks. First, 3–10 mmoles of L-Cys and 3–10 mmoles of $VOSO_4$·5H_2O were dissolved in *ca.* 40–60 ml of water, and the solution deoxygenated by bubbling nitrogen; this stream of nitrogen was maintained throughout, except when solutions or solids were being added, the pH measured, or during sampling for TLC. Ethanol (8–12 ml) containing the required amount of Hsal (3–10 mmoles) was added (t_R = 0). These solutions normally corresponded to pH ~3. Sodium acetate was added (moles = 3× those of L-Cys), and the pH adjusted to pH 5.3 or 6 with acetic acid or base. For the experiments at pH 8 and 9, solid Na_2CO_3 was added after the acetate until the required pH. The N_2 entered through one of the necks and came out through a 30 cm condenser and bubbled through 3 flasks, each containing a 100 ml aqueous solution of 3$CdSO_4$·8H_2O (6.1 g, 8.0 mmole) and 1 ml of concentrated H_2SO_4. The H_2S produced in the reaction mixture, and transferred to the flasks, precipitates as CdS (yellow). In the experiments for quantitative determination of the H_2S produced, the reaction was stopped after ~24 h by adding acid until pH ~3, and N_2 was still kept bubbling for ~30 min. In other experiments of longer duration, monitored by TLC, apparently no more H_2S was produced after ~24 h of reaction. The amount of CdS was determined by adding a known amount of standard iodine solution, and titrating the excess iodine either with a standard thiosulfate solution, or with a standard arsenious oxide solution.

L-cysteine methyl ester. Similar experiments were performed with L-CysOMe at pH *ca.* 6, 8 and 10. L-Cysteine methyl ester evolved H_2S soon after mixing, and the optical activity of these solutions was lost within *ca.* 2 h (with L-Ala this takes several days, see ESI-3†). No quantitative determination of the evolved H_2S was performed.

***S*-methyl-L-cysteine (CysSMe).** Mixtures containing L-CysSMe, $V^{IV}O$ and Hsal at pH 8 evolve a gas with the characteristic odour of a thiol. By analogy to the L-Cys system, CH_3SH would be produced from the *N*-salicylidene *S*-methyl-L-cysteinato complex, but we did not try to identify the exact nature of the gas evolved.

D,L-homocysteine, lanthione, penicillamine, methionine, cystine. No H_2S or thiol was evolved from these systems. This was normally checked by bubbling a stream of N_2 through a small column containing filter paper impregnated with a $CdSO_4$ solution. If H_2S (or presumably other thiol compounds) was produced, the filter paper would turn yellow, but this did not happen. The mixtures

were monitored by TLC using 20 × 20 cm plates divided in two 10 × 20 cm parts, each with an equal sequence of samples. One part was revealed with iodine vapours followed by a ninhydrin–collidine–copper solution, and the other part with a $PdCl_2$/HCl solution.

Detection of pyruvic acid

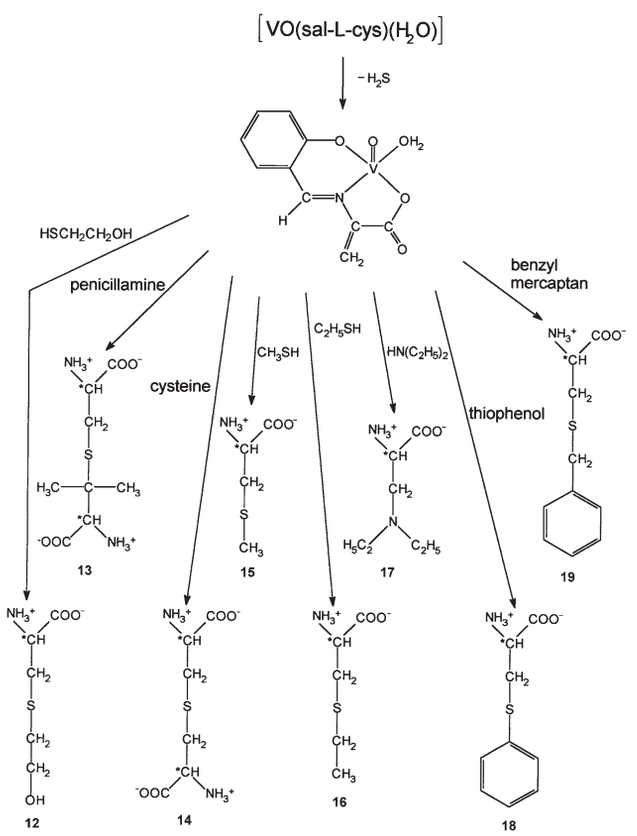
The mixture containing L-Cys, Hsal and $V^{IV}O^{2+}$, from which all H_2S had been evolved, was acidified at pH ~ 2. The precipitates formed (*e.g.* lanthionine, cystine, $VO(sal)_2$, or other), were filtered off and a solution of 2,4-dinitrophenylhydrazine in HCl (2 M) was added. The 2,4-dinitrophenylhydrazone was extracted according to the methods of Case.[35] A solid 2,4-DNP product was recrystallized from nitromethane (m.p. 213–215 °C (literature value for the pyruvic acid derivative, 214 °C). Analysis: found C, 39.9%, H, 3.3%, the product requires 40.31%, H, 3.01%.

Detection of ammonia. The presence of ammonia was detected by adding NaOH to the green acidified solutions until basic, and then blowing the ammonia formed into Nessler's reagent[36] with a stream of N_2. A brown precipitate was formed in Nessler's reagent indicating the presence of ammonia.

Racemization experiments. Equimolar solutions of 0.005 M in vanadium, of composition L-Ala or L-Met : Hsal : $V^{IV}OSO_4$, were deoxygenated, the pH adjusted to 9, and the solution kept at 20 °C. The electronic spectrum and circular dichroism were measured at intervals (see ESI-3†).

Reactions based on the dehydroalanine intermediate V (see Schemes 1 and 2)

S-Hydroxyethylcysteine **12**, (2-amino-2-carboxy-3-dimethyl)sulfide **13**, lanthionine **14**, *S*-methylcysteine **15**, *S*-ethylcysteine **16**, 2-amino-3-(diethylamino)propanoic acid **17**, *S*-phenylcysteine **18** and *S*-benzylcysteine **19**, were synthesised by the general procedure outlined below. For details see ESI-4†. The preparations were normally carried out with deoxygenated solutions and under N_2.



Scheme 2 Amino acid derivatives obtained by a Michael-type base-catalysed addition, upon addition of nucleophiles to solutions containing [$V^{IV}O$(sal–L-Cys)(X)] (X = solvent or bpy).

General procedure. $VOSO_4$ (often 3.0 mmol) was dissolved in an aqueous sodium acetate solution (*ca.* 10 mmol), and an ethanolic solution of salicylaldehyde (*ca.* 3.0 mmol) was added. Green $VO(sal)_2$ precipitated and Na_2CO_3 was added to pH 8. The appropriate nucleophile reagent was added (see Scheme 2), normally in a 5–10 fold excess relative to $VOSO_4$. Either solid L-cysteine or an aqueous solution was added, normally *ca.* 3.0 mmole, but *ca.* 12 mmole in the case of the synthesis of lanthionine **14**. The pH of the reaction mixtures was normally kept at ~8 by adding Na_2CO_3, and all reactions were monitored by TLC (see ESI-4†) using at least two different eluents. The TLC samples were applied: (i) directly, (ii) after being acidified to pH ~ 2, (iii) with acidified samples spiked with standard. The acidified samples were frozen for HPLC experiments, using a pre-column derivatization method and a fluorescence detector (see above section on HPLC methods, and ESI-4†for details). In several cases, bpy (*ca.* 3–9 mmol) was added to the reaction mixtures after the addition of the nucleophile, but no significant differences between the two experiments (with or without bpy) were noticed in the TLC and HPLC results. In some cases the products (lanthionine **14**, benzylcysteine **19**) were isolated as solids and characterised by the usual analytical methods.

EPR spectra

The X-band EPR spectra were recorded at 77 K (on glasses made by freezing solutions with liquid nitrogen), either with a Bruker ESR-ER 200D connected to a Bruker B-MN C5 ESR spectrometer and to a Bruker ESR data system, or with a Bruker ESP 300 E spectrometer.

Magnetic susceptibility

Magnetic susceptibility of polycrystalline samples of complexes (36 mg of **2**, 31 mg of **3**, 25 mg of **4** and 31 mg of **6**) were measured in the range 9–295 K using a 7-T Faraday Oxford Instruments system coupled to a Sartorius S3D-V microbalance, at 1 T and applying forward and reverse gradients of 2.5 T m^{-1}. Under these conditions the magnetisation was found to be proportional to the applied magnetic field.

Magnetic moment of the desulfydration mixture

The magnetic susceptibility of the mixtures were monitored according to the method of Evans.[37] A solution of composition Cys : Hsal : $V^{IV}O^{2+}$: OH^- = 1 : 1 : 1 : 3, 0.1 M in vanadium, was kept at 40 °C and the H_2S evolved was removed by a stream of N_2. Samples (2.5 ml) of this solution were taken, *tert*-butanol (0.2 ml) added, and the solution made up to 5.0 ml with water. The external standard was a 4% aqueous solution of *tert*-butanol. These samples were taken at 0, 2, 3, 5 and 22 h from the time of mixing, and the resonance lines of *tert*-butanol measured. A constant frequency separation of 7.7 Hz was obtained, this giving a constant μ_{eff} of ~1.7 μ_B per V atom.

Thermogravimetry and differential scanning calorimetry

Thermogravimetric (TG) and differential scanning calorimetric (DSC) curves were measured with a Setaram TG-DSC111 thermobalance, normally in the range 20–600 °C. Some TG curves were recorded on a Stanton Redcroft TG-750, connected to a Venture Servoscribe 220.

Circular dichroism and isotropic absorption spectra

CD spectra were run either on a Jasco 720 spectropolarimeter (either with a 170–800 nm or with a red-sensitive (400–1000 nm) photomultiplier), or on a Roussel-Jouan Dicrographe MK III. UV/VIS isotropic absorption spectra (UV-VIS) were run either on a Perkin Elmer λ9, or on a Cary 17 spectrophotometer.

Solid complexes 2–4, 6, 8, 9. The KBr disks or the Nujol mulls for this purpose were prepared similarly to those for IR spectroscopy but (i) the relative amounts of complex were *ca.* 50% higher, and (ii) the optical path was as low and homogeneous throughout the disk (or paste) as possible. The disk or the paste (one or two drops) was placed between two microscope slides and placed in the sample compartment in a fixed position. Depending on the complex and the intensity of the CD signal obtained, one to three of such 'samples' were used (one for the KBr disks). A first CD spectrum was run, the sample rotated ~70°–90° and another spectrum recorded; five rotations were performed for each sample (14 rotations for **4**, which had an extremely weak signal) and the corresponding CD spectrum recorded. With this type of solid sample, one does not know the position of the base line, but the correct pattern of the spectrum may be obtained if the spectrum recorded after each rotation of the sample is always approximately the same.

CD solution spectra. Before preparing the solutions, oxygen was removed from the solvents by bubbling N_2. The spectra were recorded immediately after the preparation of the solutions: the cells had their stoppers reinforced with parafilm® strips, but no special care was taken to remove oxygen from them. The cells were placed in thermostatted cell compartments.

Complexes. The complexes were normally dissolved in methanol in concentrations *ca.* 0.1–1 mM for experiments in the UV (230–400 nm), or *ca.* 1–5 mM for experiments in the visible (400–800 or 400–1000 nm). The solutions were placed in a cell (1 cm optical path) containing a small magnetic stirring bar. The spectra were run immediately, and repeated after *ca.* 15, 25, 50 and 80 min of stirring (total times after the preparation of the solution indicated). The cells were opened and a few drops of water were added. After *ca.* 10 and 20 min of stirring, CD spectra were recorded. The cells were re-opened, air gently bubbled into the solution for 10 min with stirring and a final CD spectrum recorded. Variations of this sequence were also done for the L-Cys system.

Other experiments. CD and visible spectra were also recorded in aqueous–ethanolic solutions (2 : 1) containing amino acid : salicylaldehyde : $V^{IV}O$: acetate (1 : 1 : 1 : 2), starting at pH ~ 4–5, recording the CD spectrum (400–800 nm or 400–1000 nm), and taking a sample for EPR (sample immediately frozen at 77 K). The pH was increased about 0.8 pH units, the CD spectrum recorded and another sample taken for EPR. This was normally repeated up to pH ~ 13.5. Such experiments were done with L-alanine, L-serine, L-cysteine, D-penicillamine, L-cysteine ethyl ester (EPR only) and *S*-methyl-L-cysteine (EPR only).

CD spectra of solutions containing Hsal and L-Cys, or Hsal and D-Pen (both reagents ~10^{-4} M) were also recorded. Here the corresponding sal–Cys or sal–Pen thiazolidines (tz–Cys or tz–Pen) presumably predominate.

Molecular mechanics calculations

Molecular mechanics calculations were carried out using the Universal Force Field (UFF),[38] within the CERIUS2 software.[39] This force field is parameterized for full periodic, but the minimization of a few X-ray structures revealed clearly that the default parameters found in the field were inappropriate to reproduce accurately the co-ordination spheres of the complexes. Therefore a specific set of parameters comprising the L–M bond lengths and L–M–L angles was developed empirically following a experimental procedure identical to that described in ref. 16. Partial charges were not included as they were difficult to calculate accurately and only have marginal impact on relative strain energies in metal complexes. The atom types O_R2, N_R3 and N_R2 have the same UFF properties of O_R (angular oxygen) and N_R (triangular nitrogen) respectively, in order to allow the individual assignment of the ideal distances and/or bending angles. The values of the ideal and L–M–L angles are listed in ESI-10†, Tables 1 and 2. The starting co-ordinates for the thiazolidine isomers were obtained by manipulation of the atomic co-ordinates of the available X-ray structures and organic residues (*e.g.* $-CH_2-X$,$-CH_3$ or others) were added when required. All model complexes were minimised using the conjugate gradient algorithm and a high convergence criteria with default parameters.

Density functional calculations

DFT calculations[40] were carried out using the Amsterdam Density Functional (ADF) program[41] developed by Baerends and co-workers (ADF-2002).[42] Vosko, Wilk, and Nusair's local exchange correction potential was used,[43] together with Becke's nonlocal exchange[44] and Perdew's correlation corrections.[45,46] Unrestricted calculations were carried out on the paramagnetic species. The geometry optimization procedure was based on the method developed by Versluis and Ziegler,[47,48] using the non-local correction terms in the calculation of the gradients. Full optimisation was carried out for all complexes. Full geometry optimisations without any symmetry constraints of complexes [$V^{IV}O$(sal-D-Cys)(H_2O)], [$V^{IV}O$(sal-L-Pen)(H_2O)], [$V^{IV}O$(tz–Cys)(H_2O)], [$V^{IV}O$(tz–Pen)(H_2O)], [$V^{IV}O$(sal-DHAla)(H_2O)], [$V^{IV}O$(sal-DHVal)(H_2O)] were carried out. The starting structures were based on structures of the corresponding isomers obtained from molecular mechanic calculations. In the geometry optimisations, core orbitals were frozen for V (1s, 2s, 2p), S (1s, 2s, 2p), and C, N, O (1s), while triple ζ Slater-type orbitals (STO) were used for the valence orbitals of H (1s), C, N, O (2s, 2p), and 3s, 3p, 4s, 4p, and 3d of V. A set of polarisation functions was added for H (single ζ p, d), V (single ζ.p, f) and S, C, N, O (single ζ, d, f). For the EPR calculations, all electron basis sets, consisting of uncontracted triple-ζ STO functions, augmented by polarisation functions, were used for all elements. The ZORA method[49] was used to account for relativistic effects and the spin orbit coupling. The *A* values were obtained from an unrestricted calculation and the *g* values from a spin restricted calculation with spin–orbit correction. The results of the *g* and *A* calculations are included in ESI-9†. The agreement of the *A* values with experiment is rather poor.

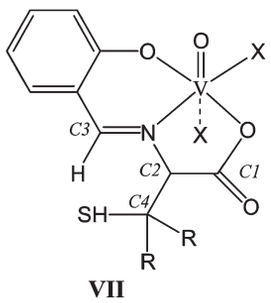
The geometries of [$V^{IV}O$(sal–D-Cys)(H_2O)] and [$V^{IV}O$(tz–Cys)-(H_2O)] obtained from the ADF calculations were optimized again using the Gaussian 98 package[50] and the IR frequencies were calculated. TD-DFT[51] calculations were performed for these two species (10 states were requested). The unrestricted B3LYP[52] formalism was adopted. The standard LANL2DZ[53] basis set, along with the associated ECP, was used for V and S, while a standard 6–31G(d) basis set[54] was used for C, O, N, and H. Graphical representations of molecular orbitals were drawn with MOLEKEL.[55]

Results and discussion

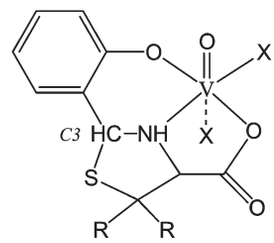
Characterisation of complexes

The formation and precipitation of the thiazolidines, and the decomposition of the amino acids activated by vanadium partly explain the difficulty in isolating complexes from mixtures containing $V^{IV}O^{2+}$, Hsal and Cys or Pen. The presence of amounts of water and/or ethanol may introduce further difficulties in establishing correct formulations. This was so with [VO(sal–L-Ala)(bpy)] **1**, corresponding to structure **IV**, which by X-ray diffraction was found to crystallise as [VO(sal–L-Ala)(bpy)]·-1/8H_2O·1/2EtOH.[7] Table 1 summarises the new complexes **2**–**9**, their C, H, N, S analyses and the formulations proposed. In some cases, the analytical results fit better with dinuclear (L)V^{V}–O–V^{V}(L) or Na[(L)V^{IV}–O–V^{V}(L)] (L = sal–aa) formulations. However, the magnetic properties measured for **2**, **3**, **4** and **6** (see below) indicate that they are monomeric, and the amount of Na^+ found was always <0.2%, so these dimeric formulations are ruled out. As mentioned, upon mixing Hsal and Cys or Pen, the corresponding thiazolidines precipitate rapidly as white powders. Presumably these may also precipitate with variable amounts of solvent as somewhat different analytical results were obtained in different batches. In Table 1 compounds **10** and **11** correspond to two of the products obtained.

The complexes may precipitate with the ligand either in the form of a Schiff base [as in **VII**: $V^{IV}O(SB)(X)$] or of a thiazolidine (as in **VIII**: $V^{IV}O(tz)(X)$). As the number of the C, H, N, S, O and V atoms in **VII** and **VIII** are identical, they may be considered tautomers, and it is not possible to distinguish them by the analytical results. This was performed by spectroscopy and by theoretical calculations.



VII



VIII

MM and DFT calculations. In the $V^{IV}O(tz)(X)$ complexes (see **VIII**), there are several stereogenic centres: the α-C (C2), the N_{amine}, the C3 and the V atom, and the *S*/*R* convention will be used for the ligand atoms (for amino acids and the corresponding SB, the D,L convention is used in this work). MM calculations were done in order to screen the possible isomers of the thiazolidine form of complexes **2** and **4** and look for the most sterically favoured ones. The results are compiled in ESI-11†. For the isomers of both [$V^{IV}O$-(tz–Cys)(X)] and [$V^{IV}O$(tz–Pen)(X)], the gas-phase strain energies follow the same trend, and the lowest energy isomers correspond to *S*,*S*,*S*,*A* configurations (*A* for the V atom).[56] These two most stable isomers were taken as models for DFT calculations (ADF program; see Experimental for details). These were carried out in order to find the lowest energy isomer obtained in the reaction of the vanadium precursor and the amino acids, namely, whether a Schiff base or a thiazolidine derivative of cysteine or penicillamine is more likely to be formed. The Schiff base form of complex **2** is more stable by 38 kJ mol^{-1} than the thiazolidine, while the difference favours the equivalent form of **4** by only 2 kJ mol^{-1}. Interestingly, another Schiff base type isomer, where the V=O and the side chain are on the same side (contrary to being on opposite sides in the preferred isomer) is only destabilised by 2 kJ mol^{-1} in the cysteine case, but by 44 kJ mol^{-1} for penicillamine. This suggests that the two complexes may exhibit different reactivity. Besides the energies, a deeper comparison between the Schiff base and the thiazolidine forms (depicted in Fig. 1) was carried out for the cysteine derivatives, by calculating the infrared (Gaussian 98) and the EPR spectra (ADF), as well as the electronic excitation energies (Gaussian 98) for both forms, and comparing them with the experimental data, as will be described in the following sections. The geometry of the desulfydrated species [$V^{IV}O$(sal–DHAla)(H_2O)] and [$V^{IV}O$(sal–DHVal)(H_2O)] were also fully optimised.

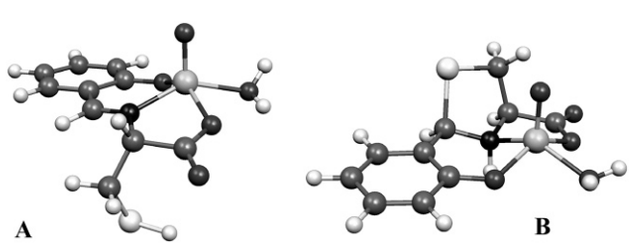


Fig. 1 Optimised structures of (**A**) the *C*-[$V^{IV}O$(sal–D-Cys)(H_2O)] Schiff base and (**B**) *A*-[$V^{IV}O$(tz–L-Cys)(H_2O)] (*R*,*R*,*R*,*C*-configuration) thiazolidine complexes.

IR spectra. All complexes present a broad band due to water centred at ~3450 cm^{-1}, less pronounced in the [$V^{IV}O$(sal–aa)(bpy)] compounds. In the IR of **2** a clear band at 2550 cm^{-1}, is due to ν(S–H). No such band is seen in complexes **3**–**9**. A medium/strong band at 1540–1560 cm^{-1}, always present, may originate from the vibration of the (Ph–)C–C(=N) bond[57] and typifies complexes derived from salicylaldehyde.[7,11,13,16,58] All complexes present very strong bands corresponding to ν(C=N) and ν_{as}(COO) centred around 1610–1620 and 1640–1650 cm^{-1} (~1690 cm^{-1} for **8** and **9**). The symmetric carboxylate stretch, ν_s(COO), probably corresponds to the medium/strong bands in the range 1335–1345 cm^{-1}. Medium to strong bands in the range 1300–1315 cm^{-1} probably correspond to ν(O–Ph). The ν(V=O) band appears in the range 955–1000 cm^{-1}. Besides these general features, some others appear or become more intense, *e.g.,* in complexes containing bpy, bands at 3070–80 cm^{-1} due to the aromatic C–H stretch.

Overall the IR of compounds **2**–**7** agree with their formulation as $V^{IV}O$ (Schiff base) complexes. However, IR spectra alone cannot rule out the presence of the ligands as thiazolidines.

The calculated IR spectra for the two tautomers of **2** differ. They were obtained from a B3LYP[51] calculation (Gaussian 98[50]). The geometry was fully optimised and the results agreed with those obtained from the ADF calculation. In the Schiff base form, the S–H stretching is calculated at 2675 cm^{-1}, a very strong V=O stretching at 1086 cm^{-1}, and an even stronger C=N vibration at 1686 cm^{-1}. Conversely, in the thiazolidine form, no such well defined and strong V=O stretching is observed in the expected energy range. These results definitely favour the Schiff base formulation. The agreement between calculated and experimental frequencies could be improved if scale factors were applied, which are available from Gaussian 98 for certain conditions and usually take values about 0.96.

Magnetic moments. The magnetic susceptibilities of **2**, **3**, **5**, and **6** were measured by the Faraday method at 1 T between 9 K and room temperature. The paramagnetic molar susceptibilities were obtained from the results after subtracting the diamagnetic contributions estimated from tabulated Pascal's constants. Fig. 2 shows the results for **2**, and similar curves were obtained with **3**, **4**, and **6**. At 20 °C the μ_{eff} values are 1.73, 1.87 and 1.70, and 1.71 μ_B for **2**, **3**, **5** and **6**, respectively, and they decrease very slightly upon cooling. If $\mu_{eff} = 2.839\ (\chi_P T + \theta)^{1/2}$ is used and/or a TIP term is included, the μ_{eff} values may become slightly smaller. The χ_P and μ_{eff} values, and their change with temperature are typical of $V^{IV}O$ compounds with a spin 1/2 per formula unit, indicating that the complexes are monomeric with no significant antiferromagnetic interactions.

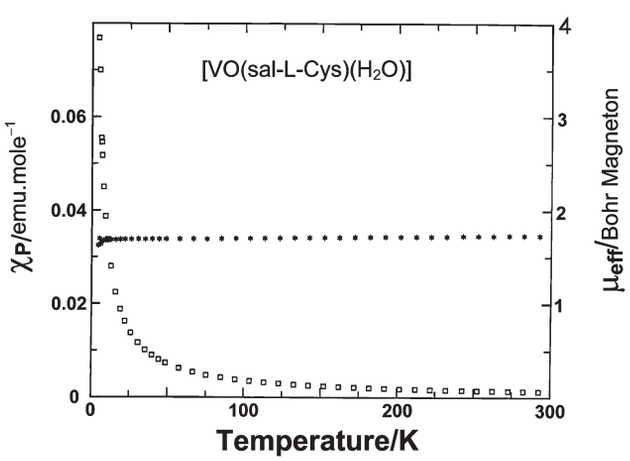


Fig. 2 Temperature dependence of the magnetic susceptibilities (□, left scale), and μ_{eff} values (*, right scale) of compound **2** in the range 9–295 K. The μ_{eff} values were calculated by the formula $2.839\ (\chi_P T)^{1/2}$.

Thermogravimetric analysis

Thermogravimetric (TG) and differential scanning calorimetric (DSC) curves were obtained for **2** and **3**. TG curves were also measured for **4** and **6**. The TG/DSC curves show trends similar to those measured for [VO(sal–GlyGly)(H_2O)$_n$].[59] For *e.g.* **2** (Fig. ESI-5†) the weight loss up to 200 °C is compatible with the loss of 1–1.2 molecules of H_2O per vanadium. The final weight, if assigned to V_2O_5, indicates a molecular weight of 315, which compares well with the 308.2 for the formulation [VO(sal–L-Cys)(H_2O)]. At

Table 2 EPR parameters obtained from the experimental EPR spectra of frozen (77 K) methanolic solutions of complexes obtained by simulation of the spectra, back-calculated $A_{\parallel}(N_{imine})$ values for each complex and $(\beta^*)^2$ values.[a]

Compound	$g_{\parallel}$	$A_{\parallel} \times 10^4/cm^{-1}$	Back-calculated$A_{\parallel}(N_{imine}) \times 10^4/cm^{-1}$	$g_{\perp}(A_{\perp} \times 10^4\ /cm^{-1})$	$(\beta^*)^2$
2: VO(sal–L-Cys)(H_2O)	1.933	164.8	**38.18**	1.977 (62.8)	0.845
3: VO(sal–L-Cys)(bpy)	1.942	161.2	**39.52**	1.977 (59.2)	0.855
6: VO(sal–D-Pen)(bpy)	1.935	159.7	**38.02**	1.974 (58.2)	0.844
8: VO(sal–L-CysSMe)(bpy)	1.950	162.0	**40.32**	1.981 (58.5)	0.877
9: VO(sal–L-CysSEt)(bpy)	1.951	162.1	**40.42**	1.981 (58.2)	0.881

[a] The square of the orbital coefficient, $(\beta^*)^2$, may be obtained from the equation: $(\beta^*)^2 = 7/6\ [(A_{\parallel} - A_{\perp})/P - \Delta g_{\parallel} + 5/14\ \Delta g_{\perp})]$, with $\Delta g_{\parallel} = 2.0023 - g_{\parallel}$ and $\Delta g_{\perp} = 2.0023 - g_{\perp}$, taking $P = 0.0130\ cm^{-1}$. The quantity $(\beta^*)^2$ corresponds to the coefficient of the vanadium $3d_{xy}$ orbital in the SOMO molecular orbital.

least three further weight losses start at ~190, ~230 and ~330 °C. Comparing these with TG (and DSC) results obtained with [VO(sal–GlyGly)(H_2O)$_n$],[59] [VO(sal–L-Ala)(H_2O)],[5] and Na[V_2O_3-(sal–D,L-Ser)$_2$],[5] the first two losses probably correspond to decarboxylation followed by oxidation of the remaining Cys moiety, and the weight loss from ~330 °C probably involves the oxidation of the benzene moiety. For **3**, **4** and **6**, the TG (and DSC for **3**) show similar trends.

EPR spectra

The EPR spectra may help to elucidate which groups co-ordinate in equatorial position in solution. Table 2 summarises results from frozen (77 K) methanolic solutions. In the spectra of **3**, **6**, **7** and **8** a second minor species could be detected corresponding to *ca.* 5–8% (**3** and **6**) and 3–5% of the main ones. Apparently these correspond to EPR parameters close to those of **2** so the minor components correspond to the [VO(sal–L-aa)(X)] (X = solvent) complexes.

Table 2 also includes the unpaired ground state (d_{xy}) orbital population $(\beta^*)^2$ estimated from the EPR spectra in methanol. The $(\beta^*)^2$ values are similar to those obtained previously from [V^{IV}O(sal–L-Ala)(X)][7,16] complexes (X = H_2O, EtOH or bpy), and indicate that the unpaired electron is mostly localised in the d orbital. The d orbital according to the ADF calculations is shown in Fig. 3 for the *A*,L isomer of complex **2**. It is essentially localized in the vanadium d_{xy} orbital (85.3%), with small π* contributions mainly from the oxygen atoms in the equatorial plane (1% $O_{carboxylate}$; 3.9% $O_{phenolate}$; 0.5% O_{oxo}), this being in agreement with the EPR results.

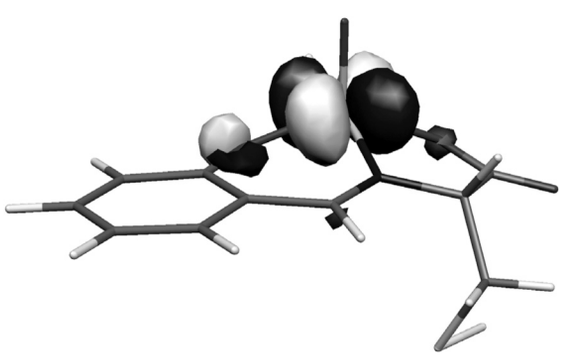


Fig. 3 The singly occupied d_{xy} orbital of complex **2**, calculated with ADF. This isomer, with *A* configuration on V and L on the cysteine α-carbon atom, is more stable than the *A*,D-isomer (enantiomer of *C*,L-) by ~2 kJ mol^{-1}.

It is well known that the EPR spectra may help to elucidate which groups co-ordinate in equatorial position in solution: for this, the so-called additivity relation[60] is often used. The $A_{\parallel}$ and $g_{\parallel}$ values obtained are in the range expected for complexes where sal–L-aa coordinates equatorially as a tridentate ligand through $O_{phenolate}$, N_{imine} and $O_{carboxylate}$, as found for other [VO(sal–L-aa)(H_2O)] and [VO(sal–L-aa)(bpy)][16] complexes (sal–aa = Schiff bases derived from the reaction of Hsal and Gly, Ala, Val, Met, Phe, Ile, *i.e.*, amino acids with no coordinating side chains). The presence of bpy gives rise to a slight decrease of $A_{\parallel}$ and $A_{\perp}$. These back-calculated $A_{\parallel}(N_{imine})$ values are among the lowest for aromatic imine donors,[61] and reflect a reduced electron-nuclear hyperfine interaction resulting from a reduced effective charge on the metal and some delocalization of unpaired spin density onto the ligands.

The EPR parameters were also obtained for *A*–[V^{IV}O(sal–L-Cys)-(H_2O)] and *C*–[V^{IV}O(tz–Cys)(H_2O)] (*R*,*R*,*R*,*C* isomer) complexes from DFT calculations (see Experimental). These are also included in Table 2. The calculated *g* values for *A*-[V^{IV}O(sal–L-Cys)(H_2O)] give better agreement with the experimental $g_{\perp}$ and $g_{\parallel}$ values than those of *C*-[V^{IV}O(tz–Cys)(H_2O)]. Constraints in the calculations, such as the functionals used,[62] spin restrictions, accounting for spin–orbit effects, may be responsible for the deviations between calculated and experimental *A* values.[16] The order of magnitude of the A_z, A_y, A_x are reasonable, but the relative error is high.

CD spectra. UV range. Circular dichroism generally gives more useful structural information on vanadyl complexes than do isotropic absorption spectra.[7,11,13] In solutions containing Hsal and L-Cys (both 10^{-4} M), where the cysteine thiazolidine (tz–Cys) presumably predominates, two intense positive CD bands are clear at ~220 and 255 nm, with a weaker band at 290 nm. A similar pattern but with opposite signs is found for solutions containing Hsal and D-Pen (see Fig. 3 in ESI-6†). As the absolute configuration of the penicillamine used is D-, to compare these CD spectra with those for corresponding solutions involving L-amino acids, the θ (or $\Delta\varepsilon$) values must be multiplied by −1. Unless otherwise specified, this factor will be implicit throughout the discussion below.

In general the $V^{IV}O$ complexes derived from Hsal possess a low-energy absorption band around 373 nm (CD) or 375 (absorption), which can be attributed to a π → π* transition originating mainly in the azomethine chromophore. For L-amino acids, these bands display Cotton effects of negative sign in the CD spectra, as found for the related zinc,[58] copper[63–65] and cobalt(II)[66] chelates. This CD band was found at 376 nm in **2**, 380 nm in **3** and 385 nm in **6**. For **2** the absorption band in H_2O/MeOH solutions was found at ~375 nm, and at ~370 nm in a MeOH solution of [V^{IV}O(sal–L-Ala)(H_2O)]. TD-DFT calculations using Gaussian 98 showed two absorption bands in this region for the Schiff base complex **2** (isomer *A*-[V^{IV}O(sal–L-Cys)(H_2O)]). The more intense is observed at 378 nm and has two main components (40 and 45%); both consist of two transitions from π* (phenol) to a π V–N bonding, also spread over benzene π*, d_{xz} orbital, and they can be considered as LMCT. A less intense band in the visible region (at ~650 nm) can be assigned to the classic $d_{xy} \rightarrow d_{xz},d_{yz}$ transition (band I); indeed, one component (40%) is $d_{xy} \rightarrow d_{xz}$, where the d_{xz} orbital belongs to a π antibonding V–O_{oxo} orbital, and d_{xy} is almost non bonding, as described in the EPR section (figure 2); the second contribution (48%) to this band arises again from d_{xy} and also ends in π V–N bonding, also spread over benzene π*, d_{xz} orbital, giving some charge transfer character to the band. The other calculated transitions, which might correspond to other d–d transitions are extremely weak (oscillator strength close to zero) and therefore were not considered (the pictures of orbitals and band assignments are given in ESI-9†).

The pattern and band intensities of the CD spectrum of solutions of **2** and **4** in methanol (*ca.* 10^{-4} M) (Fig. 4), differ from those of the thiazolidines, and are very similar to those found for [VO(sal–L-Val)(H_2O)] or [VO(sal–L-Ile)(H_2O)].[11] These intense CD bands with λ_{max} at ~255 and ~220 nm are probably associated with benzene ring π → π* and charge transfer transitions.[66,67] The imine bands are flanked by a prominent band/shoulder at 290 nm,

but this apparently corresponds to relatively weak CD activity. The CD spectra of **3** and **6** (Fig. 4), and those of **8** and **9** in methanol are similar to those of other [VO(sal–L-aa)(bpy)] complexes[11] (aa = Ala, Val, Met, Ile, Phe). Overall these CD spectral results also indicate that in the present methanolic solutions of the complexes containing Cys and Pen, the ligands are present as Schiff bases.

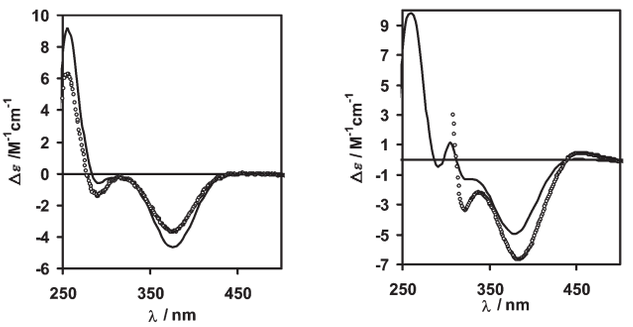


Fig. 4 CD spectra (UV range) of methanolic solutions of (A) **2** and **4**, (B) **3** and **6**. The CD spectra of **3** and **6** were multiplied by −1.

CD spectra. Visible range. In the visible isotropic absorption spectra of complexes **2–9**, band I ($d_{xy} \rightarrow d_{xz},d_{yz}$) normally appears broad, between approximately 650 and 900 nm (at least), and band II ($d_{xy} \rightarrow d_{x2-y2}$) with maxima or shoulder at ~550 nm. The CD spectra shows bands I, II and the imine band more distinctly.

Fig. 5 includes CD spectra of solids **2**, **3**, **6**, **8** and **9**. The pattern of the spectra is the same in all cases and similar to that of [VO(sal–L-Ala)(H_2O)].[7] Therefore, the structural factors that dominate the CD signals are similar, thus indicating that for all complexes in the solid state, namely those containing Cys and Pen, the ligand is the Schiff base and not the thiazolidine. Although the band pattern of **3** and **6** are similar, the λ_{max} for bands II, IB and IA differ significantly: 560, 690, 900 nm, and 560, 750, ~1000 nm for **3** and **6**, respectively.

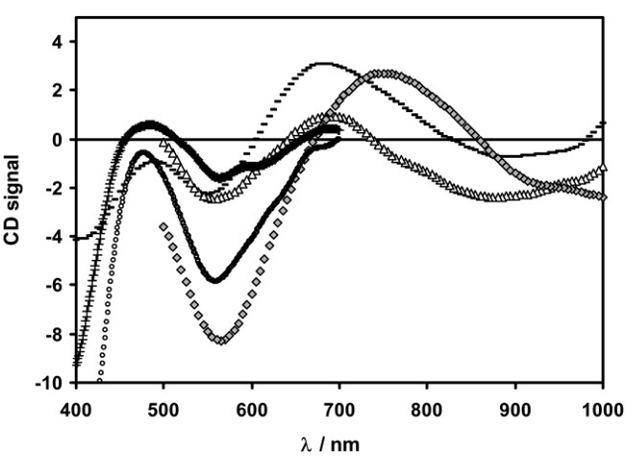


Fig. 5 Circular dichroism spectra of several complexes dispersed in KBr disks (**8**, **9**) or Nujol mulls (**2**, **3** and **4**). **2** (——); **3** (△△); **4** (◇◇); **8** (ooo); **9** (++) The spectrum of **6** was extremely weak, indicating that the amino acid partly racemized.

Fig. 6 shows spectra of methanolic solutions of the same complexes, and of [VO(sal–L–Ala)(X)] (X = H_2O, bpy) within *ca.* 5–15 min. after their dissolution. The pattern of the CD spectra in the solid state and in methanol is the same, so the coordination geometry does not change upon dissolution. It is also clear that the pattern is similar in all cases, so in these solutions all ligands coordinate similarly to sal–L-Ala.

Fig. 4 (in ESI-6†) includes the CD spectrum of **2**, and of its methanolic solution, immediately after its preparation and after several hours (*ca.* 2.2 and 3.4 h) standing in air. These spectra confirm that L-Cys did not racemize significantly within this period. The pattern

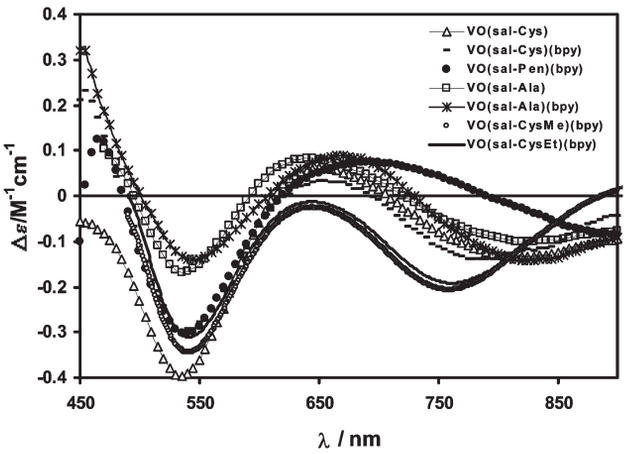


Fig. 6 Circular dichroism spectra of methanolic solutions of several $V^{IV}O$ complexes prepared in this work, and those of [$V^{IV}O$(sal–L-Ala)(H_2O)] and [$V^{IV}O$(sal–L-Ala)(bpy)] **1**. Fig. 4 in ESI-6†shows a comparison of the solid state and solution CD spectra of **2**.

of the solid state spectrum and that of the methanolic solution is the same, but on standing in air a positive band at *ca.* 440 nm appeared, indicating significant oxidation of V(IV). The band at *ca.* 440 nm is possibly due to a ligand-to-metal charge transfer transition, but as the band is superimposed on the negative imine band, its λ_{max} cannot be determined accurately.

Desulfydration of cysteine. In a stirred mixture of equimolar amounts of a $V^{IV}O^{2+}$ salt, L-Cys and an ethanolic solution of salicylaldehyde in buffered aqueous medium, ready evolution of H_2S occurred at room temperature in the pH range 5–9. This system seems to provide an analogy with the action of *cysteine desulfydrase*, a pyridoxal dependent enzyme. Bergel *et al.*[23,24] studied similar reactions in the presence of pyridoxal instead of Hsal.

Fig. 7 shows the evolution of the Cys concentration with time as obtained by TLC. At pH 3, no H_2S was detected and almost no cysteine decomposes. At pH 5.3, the desulfydration occurs. At pH 6 and at 40 °C, within 24 h the amount of H_2S formed corresponded to 50–60% of the L-Cys initially present. On increasing the pH, the rate of the H_2S evolution is accelerated, but the total amount released within the first 24 h is approximately the same in the pH range 6–9. However, at pH ≥ 8 the desulfydration proceeds very fast and after *ca.* 20 h no Cys spot is detected by TLC. The solutions after desulfydration show no optical activity. From the acidified reaction mixtures pyruvic acid was identified through its 2,4-dinitrophenylhydrazone; the presence of ammonia was confirmed by the use of Nessler's reagent.

H_2S is quite soluble in aqueous media, and at pH < 8 (though not at pH ≥ 8) small amounts of cystine and of lanthionine (which are only slightly soluble in this medium) were detected by TLC. This may explain why the H_2S measured after flushing with N_2 or argon is much less than 100% of the initial L-Cys. At pH ≥ 8 several non-identified spots were detected by TLC using a $PdCl_2$/HCl solution,

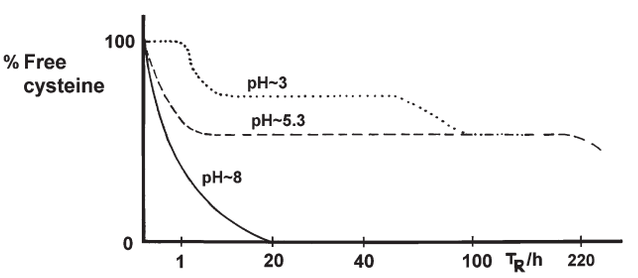


Fig. 7 Approximate change in the concentration of free cysteine, as estimated by TLC, in aqueous/ethanolic solutions containing L-Cys, Hsal and $VOSO_4$ (relative molar concentrations, 1 : 1 : 1). As cysteine may be in the form of other compounds (*e.g.*, the Schiff base, which partially hydrolyses during sample application and elution), this only gives indirect information about the cysteine that reacted to produce H_2S.

Table 3 Spin-Hamiltonian parameters calculated from the EPR spectra of frozen (77 K) aqueous : ethanolic (2 : 1) solutions containing amino acid : Hsal : $V^{IV}O^{2+}$: acetate (1 : 1 : 1 : 2),[a] and plausible equatorial donor atoms (these based on the EPR parameters and UV-Vis and CD spectra recorded). In the 3rd column, the estimated $A_{\parallel}$ values[60] are also included in brackets[b]

Species	$g_{\parallel}$	$A_{\parallel} \times 10^4$ /cm^{-1}	Predicted equatorial donor atoms
A	1.949	170 (171)	$O_{phenolate}$, $O_{aldehyde}$, $O_{acetate}$, H_2O
B	1.953	166 (166)	$O_{phenolate}$, N_{imine}, $O_{carboxylate}$, X (X = H_2O ?)
B1 (Pen)	1.951	168 (166)	$O_{phenolate}$, $O_{aldehyde}$, N_{amine}, $O_{carboxylate}$ (?)
B2 (CysOEt)	1.954	164 (162)	$O_{phenolate}$, N_{imine}, 2 × $O_{acetate}$ (?)
C	1.955	164 (165)	$O_{phenolate}$, $O_{carboxylate}$, H_2O, OH^- ?
D (Ser)	1.957	157 (159)	$O_{phenolate}$, N_{imine}, $O^-_{alcoholate}$, X (X = H_2O ?)
F (CysOEt)	~1.959	~155 (152)	$O_{phenolate}$, N_{imine}, S^-, $O_{acetate}$ (?)
E	1.959	156 (152)	$O_{phenolate}$, N_{imine}, S^-, $O_{acetate}$
E1	~1.969	~154 (152)	$O_{phenolate}$, N_{imine}, S^-, $O_{acetate}$ (?)
E2	~1.955	~157 (155)	$O_{phenolate}$, N_{imine}, S^-, H_2O (?)
G (Pen)	1.967	145 (148)	$O_{phenolate}$, N_{amine}, S^-, OH^- ?
H (Pen)	~1.966	~155 (152)	$O_{phenolate}$, N_{amine}, S^-, $O_{acetate}$ (?)
T	1.957	160 (162)	3 × OH^-, H_2O

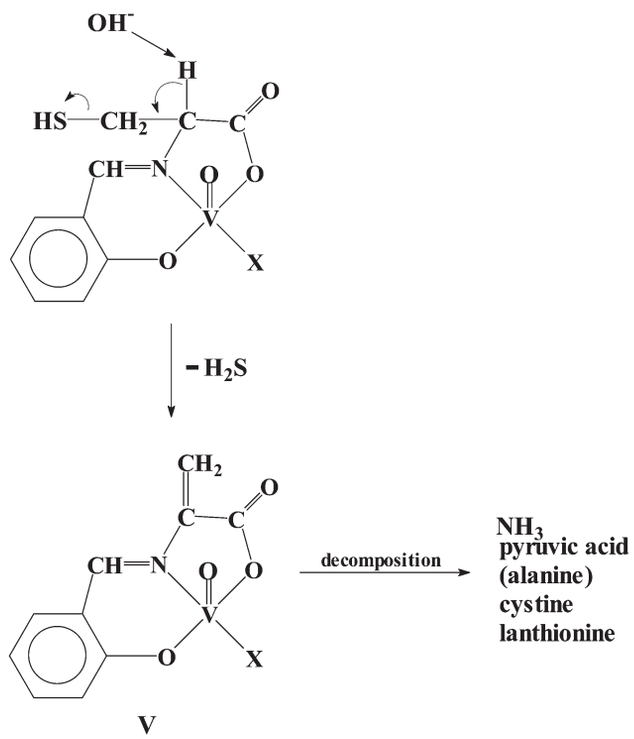
[a] The spin-Hamiltonian parameters of the species **T** were obtained by simulation of experimental spectra at pH ~ 13.5. All others were calculated following the method described by Chasteen,[60] by an iterative procedure using the corrected equations.[3] [b] To estimate the $A_{\parallel}$ values, the following equatorial contributions were considered : $O_{phenolate}$ (38.88), N_{imine} (39), N_{amine} (40.08), H_2O (45.65), $O_{aldehyde}$ (44.47), $O_{acetate} = O_{carboxylate}$ (42.1), S^- (31.92), OH^- (38.68), $O^-_{alcoholate}$ (35.32).

indicating that other sulfur-containing compounds formed. While at pH ~ 5 cystine precipitated from the mixture, identified by TLC, IR and by elemental analysis, for pH > 8 no cystine was detected by TLC. In basic medium, cystine reacts with sulfide to cysteine;[68] this could explain its non-detection.

Cystine did not desulfydrate in the presence of V(IV) salts. With V(V) salts instead of V(IV), or in solutions kept under oxygen the desulfydration of Cys is much slower. The V(V) salts catalyse the oxidation of cysteine to cystine, Cys is removed form the solution and the amount of H_2S produced decreases. Depending on the pH and on the amount of oxygen present, H_2S may not be detected.

In solutions containing $VOSO_4$, L-Cys and Hsal, in an inert atmosphere, the magnetic moment of the solutions were measured by Evans' method as the desulfydration of Cys proceeded; the μ_{eff} remained constant at 1.7 μ_B per V atom. This indicates that the desulfydration involves no valence change at the V atom, *i.e.*, it remains as V(IV).

Solutions of complex **8** and those containing the L-cysteine methyl ester (L-CysOMe) also produced H_2S, but no desulfydration was observed in the case of methionine, homocysteine, cystine (Cis) or penicillamine. β-eliminations have been extensively studied in the amino acid/pyridoxal/metal ion systems. The existence of the α-H atom is essential for reaction, and in some systems its removal has been found to be the rate-limiting step. The electronegativity of the leaving group is another important factor.[69,70] These and other observations are compatible with the mechanism outlined in Scheme 3, which involves the formation of **V**. The presence of extra groups *e.g.* $–CH_2–$, $–CH_2SR$ as in the case of methionine, homocysteine, lanthionine or cystine, hinders the β-elimination (no good leaving group may be formed). However, why no desulfydration occurs with penicillamine, neither in the presence of salicylaldehyde nor pyridoxal,[24] remains to be explained (see below).



Scheme 3 Desulfydration of cysteine and formation of the dehydroalanine complex **V**, *i.e.* the complex designated as [V^{IV}O(sal–DHAla)(X)] (X = solvent).

Spectroscopic studies of aqueous/ethanolic solutions containing amino acid, salicylaldehyde, $V^{IV}O$ and acetate (1 : 1 : 1 : 2). In the case of L-Ala, in the pH range 5.0–6.3, the EPR spectra (Fig. 5 in ESI-7†) showed two distinct signals **A** and **B**. The $A_{\parallel}$(**A**) value is compatible with V^{IV}O(sal)(X), with X = $O_{acetate}$, while the $A_{\parallel}$(**B**) corresponds to V^{IV}O(sal–L-Ala)(X) (see Table 3). The intensity ratios **B** : **A** increased with pH. Fig. 6 (see ESI†) shows a typical CD spectrum in this pH range (at pH 5.9).

For pH > 7.0 only signal **B**, which corresponds to the Schiff base complex, was detected in the EPR. As the pH was increased, this slightly shifted to lower $A_{\parallel}$ values, but the CD pattern did not change much up to pH 10.5. In ESI†Fig. 7 shows a CD spectrum typical of those recorded in the pH range 7–10. For pH > 8.3 the $|\Delta\varepsilon|$ values decreased moderately, but in the range 10.5–11.6 the CD pattern changed. This new species **C**, did not yield a clearly distinct EPR signal, but the CD signal differed (see ESI†, Fig. 8). For pH > 13 the CD signal was ~0 and the EPR corresponded to that of $V^{IV}O(OH)_3^-$(species **T**).

In the L-CysSMe system, in the pH range 5.0–12 the EPR were similar to those of the L-Ala system.

In the pH range 5.0–10 the EPR and CD spectra of the L-Ser system were similar to those of the L-Ala system (see Fig. 8 and ESI-7†Figs. 6–8). For pH > 10 a new EPR signal **D**, with significantly lower $A_{\parallel}$ values, became clear, and in the pH range 10.8–12.5 only this EPR signal could be detected. The EPR parameters of **D** are consistent with the coordination of $–CH_2O^-$ ($O_{alcoholate}$) in equatorial position. For pH > 12.5 the EPR signal shifted gradually until at pH 13.6 it corresponded to that of $VO(OH)_3^-$.

In the case of L-Cys, in the pH range 5.0–8.5 the EPR and CD spectra were similar showing the same pattern as those of the L-Ala and L-Ser systems. The binding mode of the species present is therefore the same. For pH > 8.5 the EPR showed a new signal (**E**), corresponding to a much lower $A_{\parallel}$ value (Table 3). The relative intensity of species **E** in the EPR progressively increased

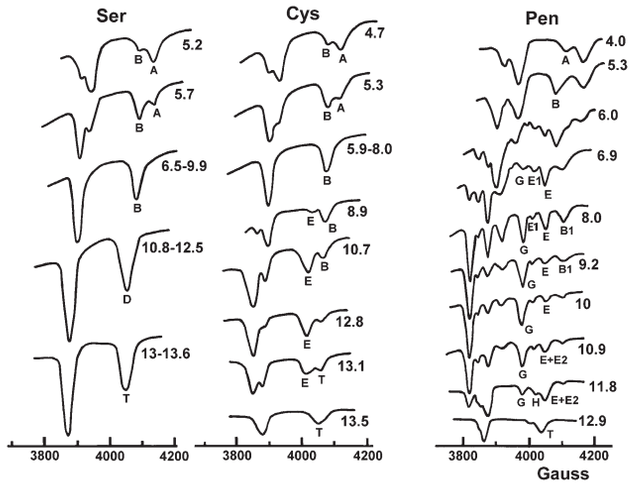
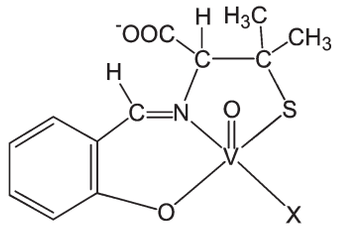


Fig. 8 EPR spectra at 77 K (high field region) of aqueous/ethanolic solutions containing amino acid : salicylaldehyde : $V^{IV}O$: acetate (1 : 1 : 1 : 2), at the pH values indicated (aa = L-Ser, L-Cys or D-Pen) and $C_{VO} \sim 0.005$ M (see also Table 3).



IX

until *ca.* pH 12, and the pattern of the CD signal also changed (see ESI-7†Figs. 6–8). These observations are consistent with the formation of the new SB complex involving equatorial coordination of $–CH_2–S^-$. The spin-Hamiltonian parameters agree with a binding mode for this complex involving $(O_{phenolate}, N_{imine}, S^-, X)_{equatorial}$ (X = H_2O or $O_{acetate}$). For pH > 12.5 the EPR signal of species **T** (corresponding to $VO(OH)_3^-$) gradually increased, this being the only signal detected at pH 13.5 (Fig. 8).

In the case of L-CysOEt (L-cysteine ethyl ester), in the pH range 5.0–6.4 the EPR showed two species: **A** + **B2**. The $A_\parallel$ values of **B2** are slightly lower than those of **B**, and its intensity increased with pH (about 50% at pH = 6). In the pH range 7–8 only **B2** was detected. Species **B2** possibly corresponds to a binding mode $(O_{phenolate}, N_{imine}, 2 \times O_{acetate})_{equatorial}$. For pH > 8 another species (**F**) was detected, this being the only distinct species recorded in the pH range 9–11.8. The $A_\parallel$ values of **F** are slightly lower than those of **E** (Cys system), and are in agreement with a binding mode involving $(O_{phenolate}, N_{imine}, S^-, X)_{equatorial}$ (X = H_2O or $O_{acetate}$; OH^- corresponds to a too low $A_\parallel$). For pH > 12 $V^{IV}O(OH)_3^-$ began to be detected, being ~60% at pH ~ 13.

In the case of D-Pen, the EPR and CD spectra recorded in the pH range 4.0–13 differed from those of all systems previously mentioned, namely those of the L-Cys system. In particular, the EPR spectra were very complex (see Fig. 8 and ESI†, Fig. 5); several species always co-existed, except at pH > 13.3 where only $V^{IV}O(OH)_3^-$ was detected.

The only species with spin-Hamiltonian parameters similar to those of **B**, was detected at *ca.* pH 5.8 ± 0.8 (see Fig. 8 and ESI†, Fig. 5). This could correspond to a $V^{IV}O$(sal–L-Pen)(X) complex, where the SB sal–L-Pen coordinates equatorially *via* $O_{phenolate}$, N_{imine}, $O_{carboxylate}$. However the CD spectra differ totally from those with similar SBs with L-Ala, L-Ser or L-Cys, indicating that the binding mode differs.

A species with EPR parameters similar to those of **E**, detected in the L-Cys system, is seen in the pH range 6–10, *i.e.* in the penicillamine system the equatorial coordination of S^- starts about 3 pH units lower than in the cysteine system. In the binary system $V^{IV}O$ + D-Pen the equatorial coordination of S^- starts about 1.3 pH units lower than in the $V^{IV}O$ + L-Cys system.[71] Several other species were detected in the EPR spectra (see Fig. 8), and the most relevant one, designated by **G**, corresponds to ($g_\parallel = 1.967$, $A_\parallel = 145 \times 10^{-4}$ cm^{-1}). This is compatible with a binding mode involving $(O_{phenolate}, N_{imine}, S^-, OH^-)_{equatorial}$, which corresponds to $A_\parallel^{est} = 148.5 \times 10^{-4}$ cm^{-1}. This could correspond to a complex such as **IX**.

In the D-Pen system, the EPR spectra showed a much greater number of species than expected for coordination of the sal/Pen ligand either only as a Schiff base or only as a thiazolidine. Moreover, the CD spectra recorded for solutions containing $V^{IV}O$, D-Pen and Hsal (1 : 1 : 1) at pH 4–6 differed totally from those of the other systems, and for pH > 7 they resemble (in pattern and λ_{max} of the bands) those recorded for the binary system $V^{IV}O$ + D-Pen. Overall the spectroscopic results in aqueous/ethanolic solution indicate that the SB complexes with binding mode $(O_{phenolate}, N_{imine}, O_{carboxylate}, X)_{equatorial}$ do not form significantly. Therefore, a possible explanation for the distinct behaviour of D-Pen in these systems, namely, the non-desulfydration of this amino acid, may be that in these solutions D-Pen is mostly present in the form of mixed-ligand complexes *e.g.* as $V^{IV}O$(sal)(D-Pen) species, the coordination of D-Pen changing with pH, or with binding modes $(O_{phenolate}, N_{imine}, S^-, X)_{equatorial}$. It is possible that $V^{IV}O$(tz–Pen)(X) (X = $O_{acetate}$, H_2O, OH^-) complexes also form.

Syntheses of the amino acid derivatives. The syntheses of several amino acids by the procedure outlined in Scheme 1 was described in the Experimental section. This is summarized in Scheme 2, where the structural formulae of the amino acids obtained are included. In most cases the nucleophile (Nu) is a thiol compound, but it may be *e.g.* an amine group as in the synthesis of **17**. The formation of amino acids **12**–**19** was confirmed by TLC and HPLC. In some cases they were isolated as in the case of **14** and **19**. As judged by the TLC and HPLC results the presence of bpy had no significant effect on the type of products nor yields.

As the ligand in the dehydroalanine complex **V** is not optically active, no enantioselectivity is expected in the reactions in Schemes 2 and 3. Besides the vanadium atom is a stereogenic centre and both *A*- and *C*-isomers[56] are expected to form in similar concentrations.[16] This was confirmed in the case of *S*-ethylcysteine (see Experimental section and ESI-7†). Further, the amino acids may racemize in these systems; this was observed for L-Ala, L-Met, L-CysOMe in this work, and for L-Asn.[18]

The success in obtaining amino acids **12**–**19** indicates that a significant concentration of the vanadium complex **VII** with the *N*-salicylidenecysteinato ligand is present in solution (a similar reaction cannot occur with the thiazolidine complex **VIII**). The reason why the desulfydration of cysteine proceeds rapidly, but no H_2S is detected in aqueous/ethanolic solutions containing Pen, salicylaldehyde and oxovanadium(IV/V) is now discussed. Three possible reasons are explored:

(i): In the cysteine system the Schiff base complex forms while in the penicillamine system the thiazolidine complexes are more stable. (ii): The desulfydrated complex **V** formed with penicillamine, [$V^{IV}O$(sal–DHVal)(H_2O)], is not stable (or its activation energy too high). (iii): Some structural or other factors are present in the penicillamine system in solution that hinders the desulfydration of this amino acid.

Calculations can shed some light on the thermodynamics of the desulfydration processes, but it must be emphasized that our MM and DFT calculations apply to the gas-phase structures, *i.e.*, entropic contributions, solvation, ion-pairing, intermolecular H-bonding and electrostatic effects were not taken into account, as is often done for coordination compounds.[72,73] Therefore, the calculations do not give definite answers and what follows should be read under this understanding. As concluded above, the Schiff base is more stable than the thiazolidine for both the cysteine and the penicillamine derivatives, but the energy is much closer for the latter one (only 2, rather than 38 kJ mol^{-1}). However, the rather similar energies of the thiazolidine and SB complexes rule out explanation (i). We can also calculate the energy differences between the Schiff base complexes and the sum of the energies of the desulfydrated species and SH_2 (ΔH_{Cys} and ΔH_{Pen}, for cysteine and the penicillamine, respectively):

$$\Delta H_{Cys} = [E(V^{IV}O(sal–DHAla)(H_2O) + H_2S) - E(\text{Schiff base})] = 55 \text{ kJ mol}^{-1} \quad (1)$$

$$\Delta H_{Pen} = [E(V^{IV}O(sal–DHVal)(H_2O) + H_2S) - E(\text{Schiff base})] = 6 \text{ kJ mol}^{-1} \quad (2)$$

Assuming that the gas phase ΔH_{Cys} and ΔH_{Pen} are good approximations of the solution ones, explanation (ii) may also be ruled out, the reaction is not driven by thermodynamic factors, as the process is endothermic, being even less favourable for cysteine, contrary to what is apparently observed.

As discussed above, in aqueous–ethanolic solutions, while the CD and EPR of the aa/sal/VO systems (aa = Ala, Ser, Cys, pH *ca.* 5–9, 1 : 1 : 1 ratio) have similar patterns, the CD and EPR spectra for the D-penicillamine/sal/$V^{IV}O$ system (1 : 1 : 1 ratio) show important differences: while with Pen for pH ≥ 5–6 the sulfur atom coordinates equatorially, with cysteine this occurs only for pH ≥ 8.8. So, the speciation differs in the two systems and complexes are formed with different binding modes. Therefore, in the penicillamine system, the much earlier coordination of the thiolate indicates a greater stability of the isomers with equatorially coordinated S^-. If in the fraction of complexes where the Pen containing Schiff base is the ligand, this coordinates through its S^- in equatorial position (*e.g.* structure **IX**), its desulfydration cannot be activated. This is certainly a relevant factor to explain the non-desulfydration of this amino acid, but as discussed above, as explanations (i) and (ii) cannot be fully accounted for, they cannot be ruled out.

Conclusions

Oxovanadium(IV) complexes with ligands derived from the reaction of salicylaldehyde with L-cysteine and with D- and D,L-penicillamine have been prepared and characterised. The spectroscopic studies in methanolic solution and in the solid state indicate that in the complexes prepared the ligands coordinate as Schiff bases. The solution structures of the complexes depend on pH and solvent, and while with L-Cys the spectroscopic results show trends similar to those of the L-Ala and L-Ser systems up to *ca.* pH 8–9, where thiolate coordination starts, the penicillamine system behaves quite distinctly, namely, thiolate coordination occurs for pH > 6.

In the presence of salicylaldehyde and $V^{IV}O$, the desulfydration of cysteine proceeds rapidly, but no similar reaction occurs with penicillamine. In the cysteine system, the *N*-salicylidenedehydroalanine–$V^{IV}O$ complex **V** is believed to form in an intermediate stage of the desulfydration, and addition of several nucleophiles to the cysteine reaction mixtures produced amino acid derivatives by a Michael-type base-catalysed addition, a result compatible with the formation of **V**.

The desulfydration may only be activated by the expected β-elimination path, modelling the action of *cysteine desulfydrase*, if the sulfur-containing amino acid is present as a Schiff base complex. However, DFT calculations for both types of tautomers (Schiff base and thiazolidine complexes) give somewhat similar energies, so no clear energetic basis for this distinct reactivity was found. Therefore, the non-desulfydration of penicillamine is probably the result of distinct speciation and/or binding modes in the cysteine and penicillamine systems in aqueous/ethanolic solutions, particularly the much greater importance of binding modes involving its S^- in equatorial position.

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