Interactions between Metal Cations and the Ionophore Lasalocid

Part 5.—A Potentiometric, Polarographic and Electron Spin Resonance Study of Copper(II)–Lasalocid Equilibria in Methanol

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> The complexation of Cu^{2+} by lasalocid and the model compound salicylic acid, AH, has been studied in methanol. The stability constants of species ACu^+ and A_2Cu were obtained from potentiometric measurements using a pH glass electrode and a copper-selective electrode, and by polarographic determinations. The stability of these copper(II) complexes was shown to be higher than that of the analogous complexes of the other transition-metal cations of the first row and very similar for lasalocid and salicylic acid. The e.s.r. spectra suggest that the structures of the species formed with the two ligands differ; the oxygen of the backbone probably participates in complexation with lasalocid. In addition, other complexes possibly involving the methoxide ion, formed in more basic media, were characterised.

Recently¹ we have studied the interaction of some first-row transition-metal cations with the ionophore lasalocid (fig. 1). We report here data on copper–lasalocid interactions. Cu^{2+} is of biological importance, and it can be used as a structural probe by virtue of its paramagnetic and spectroscopic properties. Lasalocid has been proved to mediate the transport of Cu^{2+} across weakly polar phases such as chloroform.² In deuterated chloroform, a conformation and structure for the 2:1 neutral complex of lasalocid anion with copper(II) has been proposed from the observed effects of Cu^{2+} on the ¹³C relaxation of the lasalocid anion.³

The formation of lasalocid complexes of the copper(II) ion in methanol has been studied here using potentiometry, polarography and e.s.r., with a view to assessing the relative suitability and scope of each method and obtaining more specific information.

In order to discern the role played by the salicylate moiety in the complexing power of lasalocid with regard to copper, measurements were also made with salicylic acid and 3-methylsalicylic acid.

Experimental

Materials

The specification of methanol and the preparation of tetrabutylammonium methoxide solutions were as previously described.¹ Solutions of $(ClO_4)_2 Cu \cdot 6H_2O$ were prepared in methanol. The Cu²⁺ concentration of these stock solutions being estimated in the usual way using EDTA. The methanol solutions used contained between 0.05 and 0.1% water.



Fig. 1. The structure of lasalocid.

Potentiometric Measurements

Methanolic solutions of the ligand (lasalocid or a simpler carboxylic acid) were titrated against a solution of tetrabutylammonium methoxide in the presence of copper(II) perchlorate. In order to monitor the activities of hydrogen and Cu^{II} ions during titration, three electrodes were immersed in the solution, which was maintained at 25.0 °C: a glass pH electrode (Tacussel type TB 10/HA), a Cu²⁺ crystal membrane electrode (Orion model 94-29) and a calomel electrode laboratory modified for methanolic solution, as described previously.¹

The electrodes (the two indicative ones were used alternately by means of an appropriate interface) were connected to a Tacussel Aries 10000 digital millivoltmeter, and pH standardization was carried out using De Ligny buffers.⁴ The copper-selective electrode was standardized using copper(II) perchlorate solutions of various concentrations in methanol.

Polarographic Measurements

Polarographic measurements were performed using a Tacussel apparatus consisting of a 'TIPOL' polarographic unit and an EPL 2B recording device connected to the three electrodes in the electrolytic cell. The working electrode was a dropping-mercury electrode with an out-flow velocity of 1.49 mg s^{-1} for a mercury column of 62.5 cm. The drop time was set at 2 s. The auxiliary electrode consisted of a platinum wire, and the reference electrode was analogous to that used for the potentiometry. A glass pH electrode was also placed in the cell to record the pH simultaneously.

All the polarograms were obtained at a rate of potential scanning of 2 mV s^{-1} , the movement of the recording paper having been tested beforehand. Before each run the solution was deaerated with an argon stream.

Tetraethylammonium perchlorate and tetrabutylammonium tetrafluoroborate were found to be adsorbed on the mercury drop; lithium perchlorate was accordingly selected as a supporting electrolyte. There was some complexation of lithium by lasalocid,⁵ but this was fortunately very weak compared with complexation of copper(II) so it could be neglected. All the measurements were made in a 5×10^{-2} mol dm⁻³ solution of LiClO₄ in methanol. Triton $\times 100$ was used as a maximum suppressor. Its optimal concentration was found to be 0.002%.

E.S.R. Measurements

E.s.r. spectra were recorded using a Bruker model ER 200 D spectrometer at X band ($\nu = 9.21$ GHz). 100 kHz frequency modulation was used with a phase of 90°. All the spectra were obtained at a room temperature (*ca.* 20 °C). *g* factors were determined by comparison with the e.s.r. signal of α, α -diphenyl- β -picryl hydrazil crystal (g = 2.0038).





Fig. 2. Titration of copper(II) and lasalocid or salicylic acid solutions in methanol with a tetrabutylammonium methoxide solution. 1, $2.5 \times 10^{-3} \mod \text{dm}^{-3} \operatorname{Cu}(\operatorname{ClO}_4)_2$; 2, $5 \times 10^{-3} \mod \text{dm}^{-3}$ lasalocid; 3, 3', $2.5 \times 10^{-3} \mod \text{dm}^{-3} \operatorname{Cu}(\operatorname{ClO}_4)_2 + 5 \times 10^{-3} \mod \text{dm}^{-3}$ lasalocid; 4, 4', $2.5 \times 10^{-3} \mod \text{dm}^{-3}$ salicylic acid. Curves 1–4, pH from H⁺ glass electrode. Curves 3' and 4', pCu' = $-\log c_{\text{cu}^{2+}}$ from Cu²⁺-selective electrode indications as functions of the number of base equivalents (ratio of analytical concentration of base added c_b^* to analytical concentration of copper c_{cu}^*).

Results

Potentiometry

Titrations of lasalocid–Cu^{II} ion solutions in methanol were conducted for various ratios of their initial concentrations. As regards the pH curves, trends observed here were rather different from those found for the other first-row transition-metal cations¹ and for the alkaline-earth-metal cations.⁶ Taking, for example, the ratio 2:1 as shown in fig. 2, there was for the other divalent cations a potential jump after addition of two equivalents of base [see fig. 2 of ref. (1)]; this was shown to correspond to the successive formation of 1:1 and 2:1 complexes AM⁺ and A₂M of lasalocid AH with the cation M²⁺. No potential jump was observed here for two equivalents, but a jump occurred for three equivalents of base. Therefore there must be formation of another species with an overall stability close to that of A₂M resulting either from the ionization of the phenol function or from some interaction of the methoxide ion with the Cu^{II} ion or a copper–lasalocid complex.

method ^a	r ^b	$\log \beta_{10}^{\prime c}$	$\log \beta_{20}^{\prime \ c}$	$\log \beta_{10}{}^d$	$\log \beta_{20}$
		la	salocid		
В	1:1	6.29		6.4	_
L	1:1	6.23		6.1	
В	2:1	6.16	10.12	6.5	10.7
L	2:1	6.15	10.24	6.5	10.8
В	3:1	6.11	9.95	6.4	10.5
L	3:1	6.01	10.03	6.3	10.6
		'best	values'	6.5	10.7
		salie	cylic acid		
В	2:1	5.68	9.67)	()	10.0
L	2:1	5.69	9.65	6.0	10.2
		3-methy	lsalicylic acid		
В	2:1	5.87	9.90)	()	10.4
L	2:1	5.90	9.91Ĵ	0.2	10.4

Table 1. Stability constants of 1:1 (β_{10}) and 2:1 (β_{20}) complexes of lasalocid anion, salicylate and 3-methylsalicylate with Cu¹¹ ion in methanol at 25.0 °C from potentiometric measurements (molar scale of concentrations)

^{*a*} B, H⁺ glass electrode data alone, processed using Bjerrum formation function. L, H⁺ glass electrode and Cu²⁺ solid-state selective electrode measurements, processed using Leden formation function. ^{*b*} *r* is the initial analytical ratio of ionophore to Cu^{II} ion: $c_{HA}^*/c_{Cu^{2+}}^*$. ^{*c*} Apparent constants under the conditions of the experiments: $c_{Cu^{2+}}^* = 2.5 \times 10^{-3}$ mol dm⁻³. ^{*d*} Standard formation constants.

A study of the formation of the copper methoxides in methanol was attempted. Variation of the pH of the Cu²⁺ solution on titration with tetrabutylammonium methoxide is shown in fig. 2 [curve (1)]. The shape of this curve is anomalous between the first and second equivalents of base. No precipitation was apparent, but the shape suggests that the (CH₃O)₂Cu complex is somewhat unstable in solution. A study of the electronic spectra in the visible region indicated that the true solubility at equilibrium of CH₃OCu⁺ClO₄⁻ is high (> 10⁻² mol dm⁻³), but that of (CH₃O)₂Cu is very low, of the order of 10^{-4} mol dm⁻³. Therefore, the stability constants of Cu^{II} methoxides can be determined only by using the first part of curve (1). This was done from both pH and pCu' data using Leden functions as described later. The results obtained are only qualitative and, as regards formation of (CH₃O)₂Cu, very arguable; nevertheless they demonstrate that putative formation of (CH₃O)₂Cu⁺ after ACu⁺ and A₂Cu in the course of the titration of lasalocid–Cu^{II} ion mixtures does not fit the various experimental titration curves, *e.g.* (3) and (3') in fig. 2.

It can also be seen that titration curves corresponding to salicylic acid–Cu^{II} ion mixtures (4) and (4') are rather different from (3) and (3'). As previously reported for Co^{2+} , Ni^{2+} and Zn^{2+} ,¹ between the second and third equivalents of base, a precipitate appeared, which was green in this instance. The precipitate was isolated and characterized by elemental analysis as being a complex of type $OC_6H_4CO_2Cu$, resulting from the ionization of the phenol function. No such precipitation occurred with lasalocid and, as the shape of the curve was very different, the formation of such a species could not be postulated for the ionophore.

The most reasonable assumption seems then to be the formation of a bidentate cupric complex involving lasalocid and methoxide anion, CuAMeO. The following species would then be formed successively: ACu^+ , A_2Cu , $A(CH_3O)Cu$ and $Cu(CH_3O)_2$, which is unstable in solution since it is sparingly soluble.

		potentiometry		polarography	
		$I = 0^a$	$I = 5 \times 10^{-2}$ $(ClO_4Li)^b$	$I = 5 \times 10^{-2}$ $(ClO_4Li)^b$	
$A^- + Cu^{2+} \rightleftharpoons ACu^+$	$\log \beta_{10}$	6.5	5.5	5.7	
$2A^- + Cu^{2+} \rightleftharpoons A_aCu$	$\log \beta_{20}$	10.7	9.2	9.5	
$A^- + MeO^- + Cu^{2+} \rightleftharpoons AMeOCu$	$\log \beta_{11}$	16.1	14.7	15.1	
$MeO^- + Cu^{2+} \rightleftharpoons MeOCu^+$	$\log \beta_{01}$	10	9		
$2MeO^- + Cu^{2+} \rightleftharpoons (MeO)_{\circ}Cu$	$\log \beta_{02}$	(18)	(17)		
$A^- + H^+ \rightleftharpoons AH$	$p\tilde{K}_{a}$	8.29	7.95 ^c		

Table 2. Formation constants for the complexes of lasalocid (A^-) and methoxide (MeO⁻) anions with the Cu^{II} ion in methanol at 25 °C

Estimated accuracy: ± 1 on the last figure. ^a Optimized mean values from table 1. ^b Apparent constants (concentration ratios). ^c In 5×10^{-2} mol dm⁻³ ClO₄NEt₄.

Attention was centred on the formation of ACu⁺ and A₂Cu, for lasalocid and for the model acid, as methoxide complexes have little biological interest. The stability constants of ACu⁺ and A₂Cu were obtained by two methods. pH data were processed using the Bjerrum formation function as previously described.⁶ Values thus obtained are designated B in table 1. Both pH and pCu' data (pCu' = log $c_{cu^{2+}}$) were also treated using Leden formation functions according to the principles previously used for the processing of Mn²⁺ combined with pH and e.s.r. data.¹ Values obtained in this manner are designated L in table 1. For both kinds of processing, good fits were obtained only for the first part of the titration curves, progressive deviations being observed as other species, (CH₃O)ACu for lasalocid or OC₆H₄CO₂Cu for salicylic acid, began to form. Stability constants β_{10} and β_{20} corresponding to the reactions

$$A^{-} + Cu^{2+} \rightleftharpoons ACu^{+} \tag{1}$$

$$2A^{-} + Cu^{2+} \rightleftharpoons A_2Cu \tag{2}$$

calculated from titration curves corresponding to the various initial stoichiometric ratios 1:1, 2:1 and 3:1 of ligand to copper are collected in table 1. Good agreement is observed between these data and the two different methods of processing the results.

In order to obtain data in the conditions used for the polarographic study, β_{10} and β_{20} , were also determined for lasalocid in a 5×10^{-2} mol dm⁻³ solution of lithium perchlorate in methanol using the same methods. In order to avoid any artificial decrease caused by interactions between lasalocid anion and lithium, the value used here for the pK_a of lasalocid was measured in a 5×10^{-2} mol dm⁻³ solution of tetraethylammonium perchlorate. The results are given in table 2.

The stability of the lasalocid-methoxide mixed complex was also determined in the two media using pH and pCu' titration curves corresponding to 1:1 initial stoichiometry, for which formation of A_2Cu is negligible. The stability constant β_{11} for reaction (3) is also given in table 2 along with formation constants of methoxide β_{01} and β_{02} [reactions (4) and (5)], the values of which have to be considered unreliable, as explained above:

$$CH_3O^- + A^- + Cu^{2+} \rightleftharpoons (CH_3O)ACu$$
(3)

$$CH_3O^- + Cu^{2+} \rightleftharpoons CH_3OCu^+$$
 (4)

$$2CH_{3}O^{-} + Cu^{2+} \rightleftharpoons (CH_{3}O)_{2}Cu.$$
⁽⁵⁾

Polarography

Measurements were made on lasalocid-cupric perchlorate solutions in a 3:1 ratio progressively neutralised by tetrabutylammonium methoxide. The polarogram showed only one bielectronic wave corresponding to reduction of Cu^{II} to Cu^0 whether the Cu^{II} was free (*i.e.* solvated by methanol) or complexed with lasalocid anion. The intensity of the limiting current corresponding to the complexed Cu^{2+} was a little lower than that corresponding to the 'free' Cu^{2+} and the half-wave potential was shifted to more negative values as the concentration of the added base increased. The limiting diffusion current was shown to vary linearly with both the Cu^{II} concentration and the square root of the pressure-corrected height of the mercury column, the corresponding straight lines both passing through the origin. The electrochemical process thus does seem to be a diffusion-controlled reduction of the Cu^{II} species.

Plots of log $[i/(i_D - i)]$ as a function of *E* were straight lines (*i* is the intensity of the current for potential *E*, i_D the intensity of the diffusion-limiting current), but the slopes varied from 35 mV for the reduction of Cu²⁺ alone to 64–69 mV for the reduction of Cu²⁺ in the presence of lasalocid anion A⁻. The electrochemical process was thus reversible for Cu^{II} ion alone, but only quasi-reversible for Cu^{II} ion involved in complexes with lasalocid.

A procedure of extrapolation to zero current intensity was then employed to obtain the corresponding reversible half-wave potential. Such a procedure, derived from Gellings' work,⁸ had been previously used by Gaur *et al.*⁹ and Tamamushi and Tanaka.¹⁰ The reversible half-wave potentials were obtained using the formula

$$E_{1/2}^{\text{rev}} = \lim_{i \to 0} \left(E + \frac{\mathbf{R}T}{2\mathbf{F}} \ln \frac{i}{i_{\text{D}} - i} \right).$$
(6)

The stability constants of the complexes formed between lasalocid and Cu^{II} ions could then be determined. For this purpose, only solutions corresponding to one to two equivalents of added base were found useful since in less basic solutions not all the copper was complexed and a 'free' Cu^{2+} reduction wave also appeared on the polarograms, and in more basic solutions formation of Cu^{II} methoxide would be favoured.

Results concerning the shift of the reversible half-wave potential as a function of the ligand concentration were processed using the method of De Ford and Hume,¹¹ an application to polarography of the Leden calculation method. The formula used here was:

$$\ln F_0 = \frac{\Delta E_{\frac{1}{2}}^{\text{rev}}(s) - \Delta E_{\frac{1}{2}}^{\text{rev}}(c)}{RT/2F} + \ln \frac{i_{\text{D}}(s)}{i_{\text{D}}(c)} = \ln \frac{c_{\text{Cu}}^*}{[\text{Cu}^{2+}]}$$
(7)

where s and c stand for simple and complexed Cu^{II} ion, c_{Cu}^* is the total analytical concentration of copper and $[Cu^{2+}]$ is the actual concentration.

Considering, as previously, formation of CuA⁺, CuA₂ and CuA(CH₃O), corresponding to reactions (1), (2) and (3) with the respective apparent equilibrium constants, β'_{10} , β'_{20} and β'_{11} , the Leden function is:

$$F_0 = \frac{[Cu^{2+}] + [CuA^+] + [CuA_2] + [CuAOMe]}{[Cu^{2+}]}$$
(8)

where the square brackets denote concentrations.

From the chemical equilibria, the mass balance and the electroneutrality of the system:

$$(F_0 - 1)/[A^-] = \beta'_{10} + \beta'_{20}[A^-] + \beta'_{11}[MeO^-] = F_1.$$
(9)

Extrapolation to $[A^-] = 0$ and then $[MeO^-] = 0$ yields a value of β'_{10} :

$$\lim_{[A^{-}] \to 0} F_{1} = \beta_{10}^{\prime}. \tag{10}$$

Defining

$$F_2 = (F - \beta'_{10}) / [A^-] = \beta'_{20} + \beta'_{11} [MeO^-] / [A^-]$$
(11)

then yields both β'_{20} and β'_{11} from the variation of F_2 with [MeO⁻]/[A⁻].

As [A⁻] was not known exactly, the calculations were made through successive extrapolations, starting with

$$[AH] \approx c_{\rm I}^* - c_{\rm B}^* - [H^+] \tag{12}$$

where c_1^* and c_B^* are the analytical concentrations of the ionophore and the added base. Experimental data were consistent with values thus obtained, suggesting that assumptions made about the nature of the species formed are correct. The values obtained are given in the last column of table 2. They are consistent with potentiometric data in the same medium (column 2 of table 2).

In addition, it must be mentioned that the value obtained here for the reversible halfwave potential of copper (0.178 V with respect to the saturated calomel electrode in methanol) is in good agreement with the value given by Desmarquest *et al.*¹² of the standard potential Cu^{II}/Cu⁰:0.18±0.01 V referred to the same reference electrode in methanol. The diffusion coefficient calculated using the Ilkovic equation (6.54×10^{-6} cm² s⁻¹) is for a solution of copper perchlorate alone and varies for mixed lasalocid complex solutions between 4.2×10^{-6} and 4.6×10^{-6} cm² s⁻¹.

E.S.R. Spectra

All the measurements were made with the purpose of monitoring e.s.r. spectra along with the progressive formation of the various copper complexes during the titration of mixtures of the ligand and copper(II) perchlorate in methanol against a solution of methoxide. The experimental conditions, analogous to those of the corresponding potentiometric titrations, provide the pH and the composition of the solutions analysed.

Two experimental procedures were used; first, for the lasalocid-copper ratio 2:1, independent measurements of samples corresponding to the various steps of the neutralisation process, then continuous measurements for 1:1 and 5:1 lasalocid-copper and 1:1 and 2:1 salicylic acid-copper. The latter procedure, using a circulation device, allows successive addition of base to the solution without any change of the cell orientation in the magnetic field; such changes sometimes made successive measurements using the first procedure difficult to compare reliably.

The e.s.r. first-derivative spectrum of solvated copper(II) ion in methanol is shown in fig. 3. The spectrum contains only a single broad line with a width of *ca.* 120 G centred at g = 2.185. As for the formation of mono- and di-hydroxides in aqueous solution,¹³ the formation of the methoxides corresponds to the disappearance of the signal; the decrease in the intensity of the e.s.r. signal is roughly proportional to the decrease in the concentration of the 'free' Cu^{II} ion, as calculated from the results of the potentiometric study.

Unlike the case for water,¹³ there was no solubilization of the Cu¹¹ dimethoxide complex in concentrated methoxide solution and therefore no signal corresponding to the formation of a basic complex $(CH_3O)_4Cu^{2-}$ analogous to $(OH)_4Cu^{2-}$ in concentrated hydroxide solution.

Going from acid to basic solutions in methanol, e.s.r. spectra of copper show noticeable changes corresponding to the formation of various complexes with salicylate or lasalocid anion. An example of this is given in fig. 4. The trends observed are different



Fig. 3. Experimental or recalculated first-derivative e.s.r. spectra of various Cu^{II} species formed with lasalocid LasH and salicylic acid SalH (normalized to same concentration) at room temperature. (a) Cu²⁺, (b) LasCu⁺, (c) SalCu⁺, (d) Las₂Cu, (e) Sal₂Cu, (f) Las(CH₃O)Cu and (g) (OC₆H₄CO₂)₂Cu²⁻. ▷ denotes the point used for the calculation of the g factor.

for the two ligands. If some sort of additivity of the contributions of the spectra of the various species to the resulting experimental spectra is accepted, it is possible, using previously determined stability constants, to break down, step by step, the experimental spectra and thereby isolate the spectra of the various species involved. This was done here and the internal consistency of the calculations was found to be very satisfactory. The resulting normalized spectra of the pure species in methanol at room temperature are given in fig. 3.

For salicylic acid (SalH)-copper interactions, the following species show an e.s.r. signal: SalCu⁺ with a first-derivative spectrum analogous to that observed for Cu²⁺ [a single broad line, but shifted upfield (g = 2.160) and slightly deformed] and Sal₂Cu with a four-line signal with well resolved hyperfine structure (g = 2.143, a = 59 G), analogous to that reported for the same species in dimethylformamide.¹⁴ Obviously, no signal was observed for the complex OC₆H₄CO₂Cu which precipitated, but beyond pH 9 progressive redissolution of this complex occurred and a new four-line signal was observed (g = 2.116, a = 76 G) corresponding to an as yet unidentified complex which could be (OC₆H₄CO₂)₂Cu²⁻.

For lasalocid (LasH)-copper interactions, the spectrum of LasCu⁺ was also analogous

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Fig. 4. First-derivative e.s.r. spectra for 2:1 mixtures of salicylic acid and copper perchlorate solutions $(5 \times 10^{-3} \text{ and } 2.5 \times 10^{-3} \text{ mol dm}^{-3})$ in methanol with various amounts of tetrabutylammonium methoxide, from pH 3.5 to pH 14.2 at room temperature. p denotes the beginning of the precipitation, s denotes the beginning of solubilisation of the precipitate.

to that of the solvated Cu^{2+} , but shifted upfield (g = 2.151) and more asymmetric. The signal corresponding to Las_2Cu was broader and slightly shifted (g = 2.139). A good spectrum corresponding to this species could be obtained only from a solution with a lasalocid-copper ratio 5:1, which is more favourable to the formation of this species. After pH 6 another signal was detected; this was a signal with an ill resolved hyperfine

structure; only three lines (the first, the third and the fourth) were visible; the estimated e.s.r. parameters are g = 2.142, a = 51 G. This might correspond to the species Las(CH₃O)Cu. The variation of the intensity of the fourth and largest line with the pH of the solution agrees well with the concentration changes of this species as calculated from the potentiometric data.

Discussion

Nature of the Complexes Formed

As stated above, the processing of both potentiometric and polarographic data in methanol is satisfactory assuming the formation with the two ligands AH, lasalocid or salicylic acid, of the successive complexes ACu^+ and A_2Cu . Given the noteworthy stability of these complex salts, charged complexes of the type $AHCu^{2+}$ or $(AH)_2Cu^{2+}$ need not be considered; they could be formed only in very acidic media, if they are formed at all.

The formation of AM^+ and A_2M between lasalocid or salicylic acid or, more generally, carboxylic acids and divalent cations has been previously shown to occur in the same solvent with alkaline-earth-metal cations⁶ and other transition-metal cations.¹ The Cu^{II} ion thus follows the common rule; the stability of the species formed is even stronger for this cation than for the others. However, the affinity of the Cu^{II} cation for the methoxide anion, somewhat analogous to its well known affinity for the hydroxide anion, give rise here to competition between lasalocid and methoxide anions for the ligation of the cation, which was not encountered to any such extent with the other divalent cations. This results, for lasalocid in methanol, in quasi-concurrent formation of both A₂Cu and A(CH₃O)Cu, which accordingly hinders the determination of the thermodynamic parameter pertaining to the formation of A₂Cu. Although mixed methoxide complexes themselves have no biological importance, the occurrence of such species suggests that mixed hydroxide complexes could form, under appropriate conditions. These of course could well be biologically interesting.

In basic media, the behaviour of salicylic acid differs strongly from that of lasalocid. As with Co^{2+} , Ni^{2+} and Zn^{2+} , a complex involving the salicylate dianion, resulting from the ionization of both the carboxyl and the phenol groups, precipitates. Here it was observed to redissolve in more basic medium; the resulting species could be either a mixed complex with one or two methoxide anions or more probably a charged complex ion involving two salicylate dianions. The e.s.r. spectra (fig. 3), which showed a well resolved four-line spectrum, might thus correspond to some sort of square-planar coordination of the Cu^{11} ion in a complex of the type $(OC_6H_4CO_2)_2Cu^{2-}$, well known in water.¹⁵

Stability and Structure of the Complexes

Discussion will here be restricted to the species ACu^+ and A_2Cu . For both ligands, the values of the stability constants found here, for the 1:1 and for the 2:1 complexes, are markedly higher (by 10^2 to 10^3) than those previously reported¹ for the other first-row transition-metal cations. However, most of the differences are ascribable to the first step, the formation of the 1:1 complex, the energy of the second step being only a little greater than with the other cations.

The most striking fact as regards stability constants, is the closeness of the values obtained for lasalocid and salicylic acid anions, these values being only a little lower for the latter. The constants are even closer for 3-methylsalicylate and the lasalocid anion. This would suggest that the salicylate moiety might play the main, and maybe the sole, role in the complexation of the Cu^{II} ion by the lasalocid anion. However, as shown for the alkaline-earth-metal cations,¹⁶ analogous values of ΔG do not imply structural

identity. Values of ΔH and therefore of ΔS could well strongly differ. Determination of the enthalpies of complexation of the first-row transition-metal cations by the lasalocid anion and salicylate in methanol is in progress in our laboratory, but full data are not yet available. Nevertheless, from the e.s.r. spectra obtained here and shown in fig. 3, it can be asserted that Cu^{II} ion complexes of salicylate and lasalocid anion are appreciably different, for ACu⁺, and radically different for A_2Cu . Oxygen sites other than those of the salicylate group certainly participate in the complexation of copper both in the 1:1 and in the 2:1 complexes. From the study of effects of Cu^{2+} on the relaxation of the carbons of lasalocid in the A2Cu salt in CDCl33 a structure for this complex in this solvent is suggested as follows: the two lasalocid anion molecules participate in the complexation by the salicylate part for the first, by the oxygens of the backbone O_4 , O_{6} , O_{7} and O_{8} for the second, the two molecules rapidly interchanging their role. This could also be representative of the structure of A₂Cu in methanol. Further information is awaited from an ongoing systematic study of the successive steps of copper complex formation monitored using n.m.r. techniques.

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