Protonation Equilibria of DL-3-Amino-2-hydroxypropanoic Acid and its Complexes with Cobalt(II), Nickel(II), Copper(II), and Zinc(II)

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The protonation constants of DL-3-amino-2-hydroxypropanoic acid [$H_3L^+ = {}^+H_3N{\cdot}CH_2{\cdot}CH(OH){\cdot}CO_2H$], as determined potentiometrically at 25 °C and I = 0.1 mol dm^{-3} KCl, are log K_1 13.2, log K_2 9.14, and log K_3 2.66 for the hydroxyl, amine, and carboxyl groups respectively. With cobalt(II), copper(II), and zinc(II) the main species are [M_2L_2] and [ML_2]$^{2-}$, the outstanding one being the dimer, whereas nickel(II) forms only the mononuclear complexes [NiL] and [NiL_2]$^{2-}$. The structure of the dimers probably consists of two ML units held together by bridging oxygen atoms. The ligand is bound to the metal through chelation of the aminoethanolato-moiety, while the carboxylate anion is bound *via* a water molecule of the co-ordination sphere. The complexing capacity of the present ligand is enhanced with respect to DL-4-amino-3-hydroxybutanoic acid.

THE study[1] of the equilibria of DL-4-amino-3-hydroxybutanoic acid with Cu^{II} has shown how this ligand, unlike 2-amino-3-hydroxybutanoic acid (threonine, Thr), is bound to the metal through the aminoethanolato-moiety, $H_2N{\cdot}CH_2{\cdot}CH(O^-)$. Two protons are released on co-ordination, one from the NH_3^+ and one from the OH group. There is also assistance of the carboxylato-group in the co-ordination. One of the complexes formed is a dimer, [Cu_2L_2], with bridging alcohol oxygen atoms. In order to clarify the role of the aminoethanol group as a ligand, which is also common to serine (Ser) and several compounds of pharmaceutical and biological importance such as ephedrine, adrenaline, and noradrenaline, we have now extended our researches to the ligand DL-3-amino-2-hydroxypropanoic acid (isoserine, Ise).

EXPERIMENTAL

Reagents.—DL-3-Amino-2-hydroxypropanoic acid in aqueous solution was decolourized by charcoal, recrystallized twice, and then dried *in vacuo* over P_4O_{10}, m.p. 247.7 °C (lit.,[2] 248.0 °C) (Found: C, 33.85; H, 6.95; N, 13.45. Calc. for $C_3H_7NO_3$: C, 34.30; H, 6.70; N, 13.35%). Each solution for measurements was prepared with boiled-out twice-distilled water. Hydrochloric acid solutions (*ca.* 0.12 mol dm^{-3}) were standardized against tris(hydroxymethyl)methylamine, and potassium hydroxide solutions (*ca.* 0.2 mol dm^{-3}) against potassium hydrogenphthalate. The equivalence points in the volumetric titrations were calculated by the Gran method.[3] Concentrations of stock solutions of bivalent metal chlorides containing excess of hydrochloric acid were determined by conventional analytical methods.

The starting solutions for each run were prepared by adding successively to the titration vessel exact volumes of the following solutions: (*i*) ligand, (*ii*) HCl, (*iii*) $M^{II}Cl_2$, (*iv*) 0.5 mol dm^{-3} KCl, and (*v*) sufficient twice-distilled boiled-out water to make the volume up to 25.00 cm^3. The ionic medium was 0.1 mol dm^{-3} KCl at the beginning of the titration. The concentrations of the starting solutions and the pH range of each run are in Table 1.

Apparatus.—The potentiometric measurements were made using a digital Radiometer PHM52 potentiometer with a Radiometer G 2025B glass electrode and a saturated calomel electrode (s.c.e.) as reference. The titration vessel was kept at 25.0 ± 0.1 °C by circulating water. A nitrogen stream presaturated with 0.1 mol dm^{-3} KCl solution flowed through the titration vessel. The solutions were added by means of a motor-driven Metrohm Multi-Dosimat E415 burette. The potentiometric apparatus was made automatic by applying a Hewlett–Packard HP 9810A calculator, with a peripheral HP 11264A control block and HP 11202A (I/O) and HP 11203A (BCD) interfaces, for the processing of both the voltmeter and the burette. The interfaces provide the connections between the measuring instruments and the calculator. The calculator prints the e.m.f. data only if these remain constant within ±0.1 mV for a certain time (60—90 s); similarly, it prints out a warning if the e.m.f. is not constant after a certain period of time.

TABLE 1

Initial concentrations (10^3 mol dm^{-3}) of ligand (c_L), metal (c_M), and analytical acid (c_H), and pH ranges for titrations of Ise with H$^+$ and bivalent metal ions at 25 °C and I = 0.1 mol dm^{-3} KCl

Expt. no.	Ion	c_L	c_M	c_H	pH
1	H$^+$	5.472		18.326	2.41—12.02
2		5.472		18.788	2.37—12.10
3		6.566		21.900	2.36—12.12
4		4.925		14.786	2.63—12.31
5	Co^{2+}	4.378	1.451	16.332	2.33—10.98
6		4.378	1.088	16.052	2.35—11.11
7		5.472	2.736	18.818	2.35—11.12
8		4.378	4.354	17.181	2.27—9.28
9	Ni^{2+}	5.472	2.582	18.778	2.36—9.68
10		4.378	1.421	16.530	2.31—11.08
11		4.378	1.120	15.790	2.37—11.20
12		4.378	4.306	17.816	2.23—8.39
13	Cu^{2+}	4.378	2.188	17.697	2.24—11.84
14		4.378	4.376	18.795	2.47—5.70
15		4.378	1.442	16.997	2.28—11.88
16		4.378	1.091	15.990	2.35—11.79
17		4.378	0.566	17.480	2.26—11.77
18		5.474	2.574	18.780	2.36—11.84
19	Zn^{2+}	4.378	1.445	17.446	2.25—9.62
20		4.378	1.084	16.888	2.29—9.88
21		5.472	2.581	18.850	2.39—9.15
22		4.378	4.129	17.898	2.22—8.12

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[1] A. Braibanti, F. Dallavalle, G. Mori, and E. Leporati, *J.C.S. Dalton*, 1975, 1319.

[2] Beilsteins Handbuch, Springer, Berlin, 1922, band IV, p. 504.

[3] G. Gran, *Analyst*, 1952, **77**, 661.

RESULTS

The protonation constants (Table 2) were determined by the usual graphical methods and then refined by the computer program MINIQUAD.[4]

TABLE 2

Protonation constants[a] of DL-3-amino-2-hydroxypropanoic acid at 25 °C and $I = 0.1$ mol dm^{-3} KCl. Standard deviations are given in parentheses

Acid / Group	3-Amino-2-hydroxy-propanoic	4-amino-3-hydroxy-butanoic	2-amino-1-phenyl-ethanol
OH, log K_1	13.2 ± 0.1 [b]	13.0 ± 0.1 [b,c]	11.90 [d]
NH_3^+, log K_2	9.139(21), 9.25,[e] 9.28 [f]	9.487(11), [c] 9.73 [e]	
CO_2H, log K_3	2.661(22)	3.834(12) [e]	

[a] $K_n = [H_nL]/[H][H_{n-1}L]$. [b] ±0.1 indicates the] range. [c] Ref. 1. [d] Ref. 7. [e] Ref. 6. [f] O. H. Emerson, P. L. Kirk, and C. L. A. Schmidt, *J. Biol. Chem.*, **1931**, **92**, **449**.

The titration diagrams (Figure 1) of the ligand in the presence of the metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} show that the features of the curves for Co^{2+}, Cu^{2+}, and Zn^{2+} are the same as that obtained for Cu^{2+} with DL-4-amino-3-hydroxybutanoic acid.[1] The length of the buffer zone in equivalents of base added (K[OH]) was again equal to twice the number of moles of total metal present. The buffer zone for Cu^{2+} was at a slightly lower pH than with the corresponding butanoic acid. The behaviour of $[H_3L]^+$ with Co^{2+} and Zn^{2+} was similar to that with Cu^{2+}, but the buffer zone was at $7 \leqslant pH \leqslant 8$. The titration curves for Ni^{2+} had different shapes from those of the other metals.

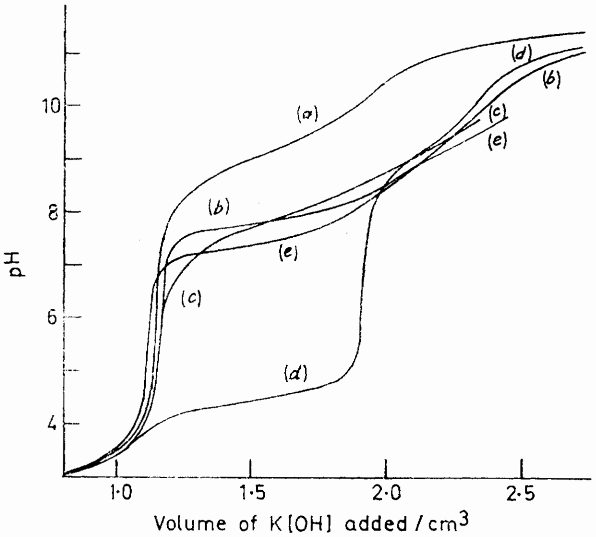

FIGURE 1 Titration curves of pH as function of the volume of K[OH] added: (*a*), ligand [experiment (2), Table 1]; (*b*) Co^{II} [(7)]; (*c*), Ni^{II} [(9)]; (*d*), Cu^{II} [(18)]; and (*e*), Zn^{II} [(21)]

The data were analysed by calculating first the $\bar{n}$ formation functions on the assumption that no protonated or hydroxo-complexes are formed in appreciable amounts, which later was shown to be valid at least in the central region of the diagrams to which the $\bar{n}$ functions refer. These functions were dependent on the total metal concentration for Co^{2+}, Cu^{2+}, and Zn^{2+}, but independent for Ni^{2+} (Figure 2).

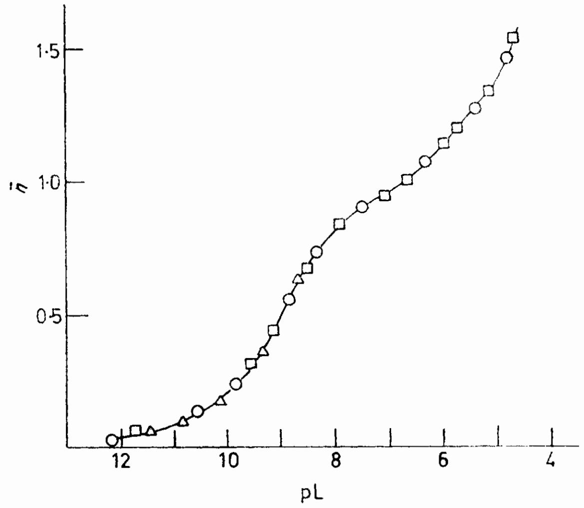

FIGURE 2 $\bar{n}$ Formation functions for the Ni^{II}–Isc system at different total metal concentrations. $c_M : c_L = 1 : 1$ (Δ) [experiment (12), Table 1], 1 : 3 (○) [(10)], and 1 : 4 (□) [(11)]

The initial set of complexes [ML], $[M_2L_2]$, and $[ML_2]^{2-}$ was tried in the program MINIQUAD,[4] as suggested by the copper(II)–DL-4-amino-3-hydroxybutanoic acid system,[1] with exclusion of $[Ni_2L_2]$ for Ni^{2+}. The refinement converged satisfactorily when the only species present were $[M_2L_2]$ and $[ML_2]^{2-}$ for Co^{2+}, Cu^{2+}, and Zn^{2+}, and [NiL] and $[NiL_2]^{2-}$ for Ni^{2+}. Other complexes, such as $[M(H_2L)]^{2+}$, $[M(HL)]^+$, $[M(HL)_2]$, $[M_2(HL)L]^+$, $[M(HL)L]^-$, $[M(OH)L]^-$, and $[M_2(OH)_2L_2]^{2-}$ were then tried one at a time. The calculations permitted the rejection of most of these complexes for each metal, except the species $[Co(H_2L)]^{2+}$, $[Cu(H_2L)]^{2+}$, and $[Zn(H_2L)]^{2+}$, present, within narrow pH limits, as up to 16, 20, and 18% of the total metal respectively, and the species $[Cu(OH)L]^-$ and $[Zn(OH)L]^-$ which never exceeded 10 and 7% of the total metal, respectively. For these hydroxo-complexes, however, the sums of the squares of the residuals differed little when the species were introduced into the calculation, and their existence is somewhat dubious. The concentrations of the species $[M(OH)]^+$ for Co^{2+}, Ni^{2+}, and Zn^{2+}, and $[Cu_2(OH)_2]^{2+}$, with constants quoted in the literature,[5] were negligible. Although the ligand has an asymmetric carbon atom, complexes $[M_2L_2]$ and $[ML_2]^{2-}$ of only one kind were considered. The sets of equilibrium constants are reported in Table 3. On the whole, the behaviour of this ligand with metals is different from that supposed by Letter and Bauman.[6]

All the calculations were made on the computer CDC 6 600 of Consorzio Interuniversitario dell'Italia Nord-Orientale, Bologna. A complete list of the experimental

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[4] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.

[5] L. G. Sillén and A. E. Martell, 'Stability Constants of Metal–Ion Complexes,' *Special Publ.*, Nos. 17 and 25, The Chemical Society, London, 1964, and 1972.

[6] J. E. Letter, jun., and J. E. Bauman, jun., *J. Amer. Chem. Soc.*, 1970, **92**, 437.

data is available as Supplementary Publication No. SUP 21685 (28 pp., 1 microfiche).*

TABLE 3

Formation constants [a] of complexes of DL-3-amino-2-hydroxypropanoic acid with Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II} at 25 °C and $I = 0.1$ mol dm^{-3} KCl. Standard deviations are given in parentheses

	Co^{II}	Ni^{II}	Cu^{II}	Zn^{II}
$\log \beta_{121}$	23.959(62)		24.120(53)	24.019(136)
$\log \beta_{101}$		9.083(15)		
$\log \beta_{202}$	20.803(22)		34.568(22)	22.779(53)
$\log \beta_{102}$	13.478(27)	13.915(77)	19.462(124)	15.821(104)
$\log \beta_{1-11}$			3.169(287) [b]	−0.496(380) [b]

[a] $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^p[L]^r$; $[OH]^q = K_w{}^q[H]^{-q}$. [b] Uncertain species.

DISCUSSION

Protonation Equilibria.—The protonation constant of the hydroxyl group of Ise, $\log K_1$ 13.2, although approximate because of its high basicity, is in fairly good agreement with the value obtained for DL-4-amino-3-hydroxybutanoic acid ($\log K_1$ 13.0),[1] and reasonably greater than that of 2-amino-1-phenylethanol ($\log K_1$ 11.90).[7] The constant of the amine group, $\log K_2$ 9.14, is in agreement with $\log K_2$ 9.49 found for DL-4-amino-3-hydroxybutanoic acid.[1] These values are lower than those of 3-aminopropanoic acid ($\log K_1$ 10.238) [8] and of 4-aminobutanoic acid ($\log K_1$ 10.556).[9] According to Clark and Perrin,[10] in fact, log K for an amine is lowered by 0.4—1.2 units by an adjacent OH group. From calorimetric data,[6] $\Delta H_2^\ominus = -42.22$ kJ mol^{-1}, and one can calculate $\Delta S_2^\ominus = 33.3$ J K^{-1} mol^{-1} which again shows the high influence, in comparison with simple amines, of the carboxyl group on the entropy term.[1] The constant of the carboxyl group, $\log K_3$ 2.66, is lower than that of 3-aminopropanoic acid, $\log K_2$ 3.551.[8] The same difference was found between propanoic acid (log K 4.874) [11] and 2-hydroxypropanoic acid ($\log K_2$ 3.86).[12]

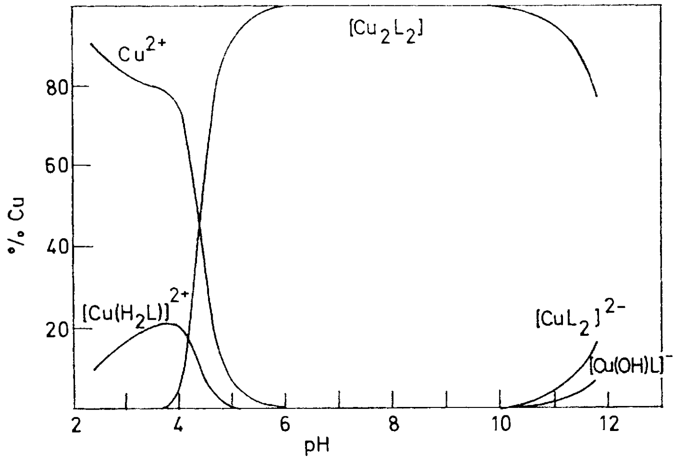


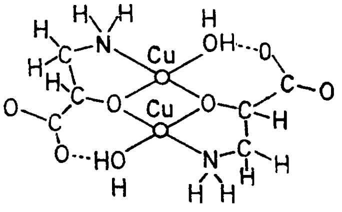
FIGURE 3 Typical distribution diagram for the Cu^{II}–Ise system. The percentages were calculated by the HALTAFALL program (N. Ingri, W. Kakalowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261) from the data of experiment (18) (Table 1). The species $[Cu(OH)L]^-$ is dubious

Metal(II) *Complexes*.—The main complexes in solutions of Cu^{II} and Ise in the range $5 \leqslant pH \leqslant 11.5$ (Figure 3) are $[Cu_2L_2]$ and $[CuL_2]^{2-}$, which have been also found in solution with the ligand DL-4-amino-3-hydroxybutanoic acid.[1]

In the present system the monomer [CuL] is not formed, due to the greater stability of the dimer ($\log \beta_{202}$ 34.57) when compared with $\log \beta_{202}$ 28.70 for the dimer of the butanoic acid analogue.[1] Also, in solutions of copper with 1,3-diaminopropan-2-ol the dimer is the only species present ($\log \beta_{202}$ 38.30).[13] The stability of the dimers can be also measured by the constants of equilibrium (1), which are log K 8.17 for Ise and log K 2.70 for the butanoic acid ligand, and log K 10.30 for 1,3-diaminopropan-2-ol.[13] A possible structure that can be assigned to this dimer consists of two adjacent chelate rings, one five-membered with oxygen bridging and one six-membered inclosing a water molecule co-ordinated to the metal. The visible spectrum of the Cu^{II}–Ise solutions shows an absorption maximum at *ca.* 645 nm, in accordance with that at 640 nm shown by the dimer of the butanoic acid ligand.[1]

$$2Cu^{2+} + 2HL^- \rightleftharpoons [Cu_2L_2] + 2H^+ \qquad (1)$$

The complexes of Co^{2+} and Zn^{2+} exhibit similar behaviour to those of Cu^{2+}, but have lower stability as expected. However, because the cobalt and zinc cations form dimers at pH *ca.* 7.5, while that of Cu^{2+} is formed at pH *ca.* 4.5, the question arises of whether the equilibria involving Co^{2+} and Zn^{2+} can be interpreted on a different basis from that used for Cu^{2+}. All these equilibria in the first buffer zone could be interpreted according to the hydrolytic equilibria (2) with formation



$$2M^{2+} + 2H_2L + 2H_2O \rightleftharpoons [M_2(OH)_2(HL)_2] + 4H^+ \qquad (2)$$

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* For details see Notices to Authors No. 7 in *J.C.S. Dalton* 1975, Index issue.

[7] R. F. Jameson and W. F. S. Neillie, *J. Chem. Soc.* (*A*), 1965, 2391.

[8] M. May and W. A. Felsing, *J. Amer. Chem. Soc.*, 1951, **73**, 409.

[9] J. J. Christensen, R. M. Izatt, D. L. Wrathall, and L. D. Hansen, *J. Chem. Soc.* (*A*), 1959, 1212.

[10] J. Clark and D. D. Perrin, *Quart. Rev.*, 1964, **18**, 295.

[11] H. S. Harned and R. W. Ehlers, *J. Amer. Chem. Soc.*, 1933, **55**, 2383.

[12] R. P. Bell, 'The Proton in Chemistry,' Cornell University Press, Ithaca, New York, 1959, p. 64.

[13] R. Näsänen, P. Tilus, H. Järvinen, and I. Komsi, *Suomen Kem.*, 1970, **B43**, 154.

of hydroxo-complexes; the results of the calculations should be equivalent. If equation (2), however, is compared with the assumed equation (3), one can see

$$2M^{2+} + 2H_2L \rightleftharpoons [M_2L_2] + 4H^+ \qquad (3)$$

that (3) corresponds to a one-step reaction, with the protons dissociating as soon as one ligand molecule approaches the metal, whereas equation (2) is a two-step

$$2M^{2+} + 2H_2L \rightleftharpoons 2[M(HL)]^+ + 2H^+ \qquad (4)$$

$$2[M(HL)]^+ + 2H_2O \rightleftharpoons [M_2(OH)_2(HL)_2] + 2H^+ \qquad (5)$$

reaction with two stepwise constants whose ratio should be different for different metals.

The two distinct steps have been observed for example in solutions of Cu^{II} with several ligands.[14] The titration curves of Ise indicate a one-step reaction, with parallel behaviour for Co^{2+}, Cu^{2+}, and Zn^{2+}. On the other hand the present ligand forms the solid complex $[CuL]\cdot3H_2O$, which remains as [CuL] when heated at 170 °C,[15] and complexes $[Cu_2Br_2L_2]$ [16] and $[ZnBr_4L_4]$ [17] (L^- = *NN*-diethylaminoethanolato) have been shown to exist in crystals. We assume, therefore, that equation (3) is the more likely interpretation of the equilibria in the solutions of Co^{2+}, Cu^{2+}, and Zn^{2+}, and that the main species are the same for the three metals.

The behaviour of Ni^{2+} is different from that of Co^{2+} in the sense that no dimers are formed. No example of this sort has ever been observed. The only protonated complexes $[M(H_2L)]^{2+}$ (M = Co, Cu, or Zn) present in appreciable amounts have constants {log K for $M^{2+} + H_2L \rightleftharpoons [M(H_2L)]^{2+}$} which are comparable with those of the corresponding acetates.[5]

It is interesting to compare the co-ordination of the ligands Ise and DL-4-amino-3-hydroxybutanoic acid, which are bound through chelation with the ionized aminoethanol group, with the co-ordination of Ser and Thr which are bound through chelation with the α-amino-acid residue. This different mode of co-ordination is surprising when the free-energy changes of the reactions with Cu^{II} are considered; values of $\Delta G^{\ominus}$ for reaction (6)

$$2Cu^{2+} + 2L^{2-} \rightleftharpoons 2[CuL] \qquad (6)$$

are −89.62 for Ser and −90.76 kJ mol⁻¹ for Thr,[6] and for reaction (7) the values are −197.30 for 3-amino-2-hydroxypropanoic acid and −163.79 kJ mol⁻¹ for 4-amino-3-hydroxybutanoic acid.[1] The comparison shows

$$2Cu^{2+} + 2L^{2-} \longleftarrow [Cu_2L_2] \qquad (7)$$

that co-ordination with the aminoethanolato-group is thermodynamically preferred. Dimerization is not an essential factor, as shown by $\Delta G^{\ominus} = -147.95$ kJ mol⁻¹ for 4-amino-3-hydroxybutanoic acid[1] [reaction (6)]. One must conclude that Ser and Thr, notwithstanding the thermodynamics, prefer chelation through the α-amino-acid residue.

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[14] A. E. Martell, S. Chabereck, jun., R. C. Courtney, S. Westerback, and H. Hyytiainen, *J. Amer. Chem. Soc.*, 1957, **79**, 3036.

[15] E. Fischer and H. Leuchs, *Chem. Ber.*, 1902, **35**, 3787.

[16] A. Pajunen and M. Lehtonen, *Suomen Kem.*, 1971, **B44**, 200.

[17] W. Haase, R. Mergehenn, and R. Allmann, *Acta Cryst.*, 1975, **B31**, 1184.