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268. Metabolites of Microorganisms

144th communication¹)

The Crystal and Molecular Structure of 3-Hydroxy-4-methylproline

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(4. X. 74)

Summary. A new amino acid isolated from the acid hydrolysate of the antibiotic Echinocandin B is shown by X-ray analysis to be 2,3-*trans*-3,4-*cis*-3-hydroxy-4-methylproline. The crystals are orthorhombic, $a = 5.633$ Å, $b = 8.760$ Å, $c = 14.314$ Å, $Z = 4$, space group $P2_12_12_1$. The structure was determined by direct methods and refined by least-squares analysis to a final R value of 0.032 for 532 reflections. The molecule exists as a zwitterion forming one O—H...O and two N—H...O hydrogen bonds.

Introduction. – A new amino acid isolated by *Benz et al.* [1] from an acid hydrolysate of the antibiotic Echinocandin B was assumed to be 3-hydroxy-4-methylproline on the basis of spectral data, especially the proton magnetic resonance spectra of the compound. The purpose of the present work was to confirm this structure independently by X-ray analysis and to establish its configuration.

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¹) 143rd Commun. see [1].

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Experimental Part. – Suitable crystals were obtained by vapour diffusion of ether into an aqueous ethanol solution. Cell dimensions and other information are summarized in Table 1. Intensity data in the range $\theta < 26°$ were collected on the Y-290 four-circle diffractometer (*Hilger & Watts Co.*) by the $2\theta/\omega$ scanning technique using MoKα radiation ($\lambda = 0.71069$ Å) monochromatized by reflection from graphite. Backgrounds were measured on both sides of the reflection peaks for $^1/_4$ of the scanning time. *Lorentz* and polarization corrections were applied in converting these intensities into structure factors and normalized structure factors, but absorption corrections were regarded as unnecessary.

Table 1. *Crystallographical data*

Molecular formula	$C_6H_{11}NO_3$	Cell dimensions	$a = 5.633$ Å
Molecular weight	145.2		$b = 8.760$, $c = 14.314$
Melting point	265° (decomp.)	Z	4
$[\alpha]_D$	$-21°$ ($c = 0.425$, H_2O)	F(000)	312
	$+10°$ ($c = 0.40$, 5N HCl)	Volume of unit cell	706.3 Å^3
Crystal size	0.40 × 0.15 × 0.10 mm	Density	calc. 1.366 g/cm^3
Space group	$P2_12_12_1$	Number of measured reflections	833

Table 2. *Final positional and temperature factor parameters* (and their e.s.d.'s) *of non-hydrogen atoms* (×10^4). The temperature factors take the form

$$\exp\,[-\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl]$$

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(3)	2813(6)	7400(4)	1610(2)	378(14)	80(4)	35(2)	−138(14)	−61(9)	4(4)
O(2)	−1224(6)	3418(3)	247(3)	194(10)	70(4)	78(2)	−25(12)	−72(9)	−39(5)
O(1)	−2728(5)	5715(3)	−66(2)	131(9)	103(4)	52(2)	24(13)	−19(7)	48(5)
N	3151(6)	4417(4)	584(2)	93(10)	75(4)	32(2)	12(11)	1(7)	−17(5)
C(6)	2083(14)	5324(7)	3111(4)	493(27)	140(9)	30(2)	−119(28)	−37(15)	4(8)
C(5)	3573(10)	4127(6)	1613(3)	270(19)	122(7)	38(3)	93(22)	−45(11)	28(7)
C(4)	1527(8)	4926(5)	2098(3)	239(16)	88(5)	25(2)	−65(19)	2(9)	9(6)
C(3)	1020(8)	6289(4)	1463(3)	202(14)	64(5)	27(2)	−11(17)	2(10)	−3(5)
C(2)	1221(7)	5592(4)	481(2)	120(12)	54(5)	23(2)	15(16)	8(8)	8(5)
C(1)	−1099(7)	4820(4)	185(3)	132(13)	85(5)	24(2)	−4(15)	19(9)	−3(6)

Table 3. *Final parameters of hydrogen atoms*

	x	y	z	B
H(Na)	0.449(9)	0.479(6)	0.033(3)	4.2 Å^2
H(Nb)	0.280(9)	0.340(5)	0.026(3)	4.2
H(2)	0.168(6)	0.641(4)	0.001(2)	1.2
H(3)	−0.053(10)	0.675(6)	0.156(3)	5.6
H(0)	0.270(10)	0.807(6)	0.121(4)	3.6
H(4)	0.005(8)	0.416(5)	0.208(3)	3.8
H(5a)	0.512(10)	0.461(7)	0.180(4)	4.9
H(5b)	0.364(12)	0.292(7)	0.175(4)	6.0
H(6a)	0.373(12)	0.621(7)	0.318(4)	7.6
H(6b)	0.087(13)	0.567(8)	0.338(4)	5.7
H(6c)	0.227(12)	0.439(8)	0.346(4)	8.2

Structure analysis. – The structure was solved by direct methods [2] with the help of program MULTAN [3]. An initial lack of success was traced to an unsuitable choice (by the program) of the enantiomorph determining reflection. Subsequent tangent refinement, based on a more suitable choice, was carried out for 16 possible phase combinations, but only eight distinct solutions were obtained, the remaining eight being related by an origin shift. The solution with the lowest R-factor led to an E-map in which the positions of all 10 non-hydrogen atoms could be recognized. Coordinates obtained from the E-map were refined by least-squares analysis (three cycles full-matrix refinement with isotropic thermal parameters) to give an R factor of 9.5%. At this stage all 11 hydrogen atoms were easily recognized in an $(F_o - F_c)$-synthesis based only on low-order reflections ($\sin \theta/\lambda < 0.4$). Further refinement reduced the R-factor to 3.2% for the 532 reflections with $F > 3\sigma(F)$. In the final cycles all 21 atoms were included, with anisotropic thermal parameters for non-hydrogen atoms and isotropic ones for hydrogens. A final $(F_o - F_c)$-synthesis based

Table 4. *Observed structure factors.* Every *hkl* is followed by values of $10. F_0$ for (hkl) (hk, l + 1) (hk, l + 2) etc.

```
0  0  2* 816,   0, 588,   0,1435,   0, 214,   0, 199,   0,  44,   0,  96,   0,  41*  0  1  1*   0,
0  1  2* 984, 550, 490, 110, 575, 368, 382,  71, 167,   0, 215, 153,   0,   0,   0,  18,
0  2  0*1239,   0, 414, 221, 744, 438,  48, 213, 217,  76, 203,  92, 173,  95,  43,  35,  83,   0,
0  3  1* 138, 206, 816, 228,  84, 238, 130, 267, 349,  58,  47,  39,  36,  52,  78,   0,
0  4  0* 196, 156, 350,  54,  65, 146,   0,  35, 232,  19, 101, 117,  62,  19,  51, 134,  30,
0  5  1* 248,  76, 368,   0, 168, 194, 214,  39,  41, 157,   0, 117,   0,  52, 106*  0  6  0* 126,
0  6  1*  15,   0,  98, 231,  37,  96,  27,  70, 104, 182,   0, 171,  29,  54*  0  7  1* 261,  67,
0  7  3*  74,  81, 182,   0,  64,   0,  55, 215,   0, 105,   0*  0  8  0*   0,   0, 254,  74, 124,
0  8  5* 141, 132,  77, 109,  84,   0,  86*  0  9  1*  77,  23, 249, 241, 136, 103,  55,  47, 146,
0 10  0* 167, 107, 134,  49,  15, 103,  38*  1  0  1* 351, 975, 123,  51, 312,  43, 248, 263, 209,
1  0 10* 134, 240, 350,  40,  18,  71,  52,  21*  1  1  0* 842, 614, 912, 354, 572, 333, 234, 272,
1  1  8* 647, 243, 266, 117, 200,  83,  54,  72,   0,  80*  1  2  0* 310, 258, 287, 126, 764, 180,
1  2  6* 362, 109, 197,  21, 267, 196, 116,  47, 133,  78,   0,  46*  1  3  0* 381, 340, 381,  98,
1  3  4* 185, 262, 154, 163, 349,  93,  75, 249, 217,   0,  43,  14,  67*  1  4  0* 387, 548, 235,
1  4  3* 171,  47, 361, 126, 101, 169, 106, 157, 151,  57,  77,  37,  62,  48*  1  5  0*  23, 155,
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1  9  9*  67*  1 10  0*   0,  86,  73,  33,  91,  28,  30*  2  0  0*1078, 558, 184, 226, 166, 193,
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2  4  2* 354, 160, 233, 130, 133, 219, 165,  76, 104,  56, 128,  69,  30,   0*  2  5  0*  70, 142,
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2  6  3* 393, 271, 163, 247,  96,  47,  55,  53,  46, 112,  96*  2  7  0* 179, 173, 218, 122, 157,
2  7  5* 199,   0,  67,  71, 102,  60, 107,  60*  2  8  0*  24,  27, 154, 171, 154,   0,  70,  48,
2  8  8* 113,  18,  91*  2  9  0*  57,  56,  75,  56,  65,  48,  46,  10,  72*  2 10  0*  80,   0,
2 10  2*  49,   0,  26*  3  0  1*   0,  40, 124, 207, 244, 135,  98, 170,  28,  82, 289, 157,   0,
3  0 14* 149,  47*  3  1  0* 141,  83,  31, 158,  89, 233,  78,  33,  84, 170, 100, 120,  62,  84,
3  1 14*  87,   0*  3  2  0* 150, 598, 262, 366, 302, 233,  79, 184, 171,  24, 191, 117,  60,   0,
3  2 14*  74,  71*  3  3  0*  38, 222, 347, 220, 172,  61,  42, 126, 181, 113,  79, 104, 151,  48,
3  3 14*  38,  43*  3  4  0* 337, 228, 194, 220, 105, 127, 130,  34, 287,  99, 191,  94,  68,  35,
3  4 14* 105*  3  5  0* 295, 182, 151,  47, 292, 231, 262,  80, 104,  82,  32,  34,  68,  69,
3  6  0*   0, 212,  91, 164, 157,  49, 134, 150, 148,  35,  96,   0,  72*  3  7  0* 159, 207, 210,
3  7  3*  63, 213, 154, 102,  95,  57,  53,  57,  53*  3  8  0*  59, 123,  33,  14,  47,  89,  34,
3  8  7*  23,  46,  55*  3  9  0*   0,  10,  80,  40,  26,  24,  56*  4  0  0* 356,  94, 160, 267,
4  0  4*  54, 158,   0,  69,  47, 289, 150,  76, 132,   0,   0*  4  1  0*  36, 199, 316, 212, 120,
4  1  5* 172, 249, 109, 222,  67, 138, 165,  40,  53,  38*  4  2  0* 196,  53,  75,  53, 118, 104,
4  2  6*  75, 131,  78, 119,  97,  36,  22,  92,  32*  4  3  0* 275, 139,  96, 189,  42, 144, 153,
4  3  7* 124,  99, 155,  48, 124,  80,  58*  4  4  0* 111,   0,  90, 101, 193, 170,  93,  96,  42,
4  4  9*  59, 147,  59, 108*  4  5  0* 110,  97, 103, 144,  47,  51, 120, 161,  84,  37,  61,  72,
4  6  0* 194,  92, 114,  69, 185, 105, 130,  28, 102,  54,  94*  4  7  0*  31,  65,  49,  97,  80,
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5  2 10*  68,  31*  5  3  0* 117,  58,  35,  95, 103,  49,  47,  70,  71,  38,  23,  41,
5  4  0*  29,  13,  83, 106,  88,  63, 110,   0,  99,  66,  47*  5  5  0*  81,  58,  99,  47,  58,
5  5  5*  54,  94,  65,  47,  28*  5  6  0*  35,  52,  32,  43, 115,   0,  10,  24*  5  7  0* 144,
5  7  1*  43, 147,  66,  73*  6  0  0*  27,  26,  86, 174,  47, 130, 124, 167, 150*  6  1  0* 117,
6  1  1*  74,  58,  84,  40,  76, 106,  53,  40*  6  2  0*  37,  75, 111,  26,  59,  47,  53, 160,
6  2  8* 144*  6  3  0*  20, 114,  41,  30,  40, 109,  35,  46*  6  4  0*  31,  63,  50,  46,  36,
6  4  5*  33,   0*  6  5  0*  40,  77,  54,  58,
```

on the 225 reflections with $F > 2\sigma(F)$ and $\sin\theta/\lambda < 0.4$ ($R = 2.5\%$) showed no electron density greater than 0.08 e/Å^3. The final coordinates and thermal parameters are listed in Tables 2 and 3 with their estimated standard deviations in parentheses. Observed structure factors are shown in Table 4.

Description of the structure. – The X-ray analysis confirms the proposed structural formula and establishes the configuration. The conformation of the molecule is evident from the stereoscopic view shown in Fig. 1. The chemical evidence,

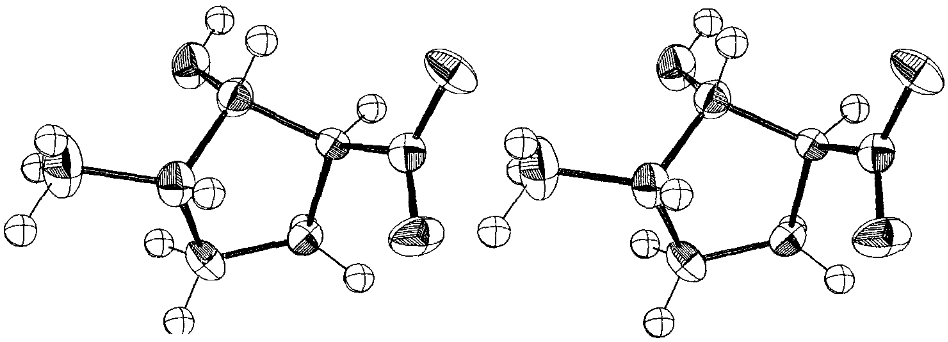

Fig. 1. *A stereoscopic view of the molecule*

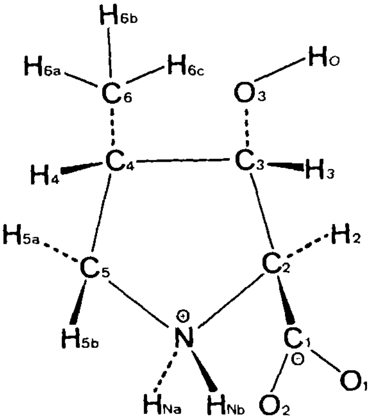

Table 5. *Bond distances* (standard deviations in parentheses)

C(1)–O(1)	1.259(5) Å	N–H(Nb)	1.02(5)
C(1)–O(2)	1.233(5)	C(2)–H(2)	1.02(3)
C(1)–C(2)	1.531(5)	C(3)–H(3)	0.97(6)
C(2)–N	1.504(5)	O(3)–H(0)	0.82(5)
C(2)–C(3)	1.536(5)	C(4)–H(4)	1.07(5)
C(3)–O(3)	1.418(5)	C(5)–H(5a)	1.01(6)
C(3)–C(4)	1.528(6)	C(5)–H(5b)	1.07(6)
C(4)–C(5)	1.517(7)	C(6)–H(6a)	1.21(7)
C(4)–C(6)	1.523(7)	C(6)–H(6b)	0.84(7)
C(5)–N	1.514(6)	C(6)–H(6c)	0.96(7)
N–H(Na)	0.90(5)		

Table 6. *Bond angles* (standard deviations in parentheses)

O(1)–C(1)–O(2)	126.8(4)°	C(2)–C(3)–O(3)	110.9(4)
O(1)–C(1)–C(2)	115.2(3)	C(2)–C(3)–C(4)	102.7(3)
O(2)–C(1)–C(2)	117.9(4)	O(3)–C(3)–C(4)	108.4(4)
C(1)–C(2)–N	110.0(3)	C(3)–C(4)–C(5)	103.3(4)
C(1)–C(2)–C(3)	111.4(3)	C(3)–C(4)–C(6)	115.2(4)
N–C(2)–C(3)	103.7(3)	C(5)–C(4)–C(6)	112.7(5)
C(2)–N–C(5)	108.8(3)	C(4)–C(5)–N	104.4(4)

especially the positive optical rotatory shift on addition of HCl to the aqueous solution, suggests the absolute configuration at the α-carbon atom to be S, in which case the compound, including specification of chirality, is 3(*S*)-hydroxy-4(*S*)-methylpyrrolidine-2(*S*)-carboxylic acid.

Table 7. *Torsion angles*

N–C(2)–C(3)–C(4)	–34.7°	C(3)–C(2)–N–C(5)	15.0
C(2)–C(3)–C(4)–C(5)	41.7	O(2)–C(1)–C(2)–N	13.5
C(3)–C(4)–C(5)–N	–32.3	C(1)–C(2)–C(3)–O(3)	–160.8
C(2)–N–C(5)–C(4)	10.7	O(3)–C(3)–C(4)–C(6)	47.6

Bond distances, angles and torsion angles are listed in Tables 5, 6 and 7. The molecule is a zwitterion, and the methyl and the hydroxyl groups are both *trans* to the carboxyl group. The values of the torsion angles in Table 7 indicate that the conformation of the five-membered ring can be described as a 'twist' form with an approximate twofold axis through the nitrogen atom. The carboxyl and hydroxyl groups can be described as *pseudo* axial, the methyl group as *pseudo* equatorial to the ring. The conformation about C(1)–C(2) is shown in Fig. 2. It is apparent that the nitrogen atom lies fairly close to the plane formed by the four atoms O(1), O(2), C(1) and C(2), as has been observed in several previous X-ray analyses of amino acids. Bond distances in the ring tend to be slightly longer than those observed in a recent neutron

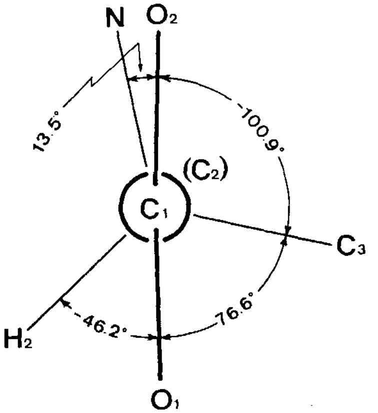

Fig. 2. *Newman projection along C(1)–C(2) bond*

diffraction study of 4-hydroxy-L-proline [4]. The two C–O bonds of the COO^- group are significantly different, whereas they are almost equal in 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline [5] and 4-hydroxy-L-proline [4]. The C–OH distance of 1.418 Å is slightly longer than in hydroxyproline (1.403 Å) but shorter than in the dihydroxy compound (1.424, 1.433 Å). The bond angles around C(1) are normal, but the others are slightly different from those reported for proline and substituted prolines, possibly because of changes in the conformation of the five-membered ring and in the configuration of the substituents. Fig. 3 shows the molecular packing and hydrogen bonding scheme viewed along the *a*-axis. The hydrogen bond distances are listed in Table 8. The hydrogen bond N–H(Na) ... O(1) contributes to the stabilization of the crystal structure in the *a* direction. The other two hydrogen bonds, O(3)–H(O) ... O(1) and N–H(Nb) ... O(2), join the molecules along *c* and *a*. There is no important contact between molecules in the *b* direction.

Table 8. *Hydrogen bond distances* (The e.s.d.'s are in parentheses)

X–H...Y	Translation for atom Y (*a*, *b*, *c*)	Symmetry operation for atom Y	Distance (X...Y)	Distance(H...Y)
N–H(Na)...O(1)	(1, 0, 0)	x, y, z	2.747(4) Å	1.85(5) Å
N–H(Nb)...O(2)	(0, 0, 0)	$^1/_2$+x, $^1/_2$–y, –z	2.776(5)	1.84(5)
O(3)–H(O)...O(1)	(0, 1, 0)	$^1/_2$+x, $^1/_2$–y, –z	2.775(4)	1.97(5)

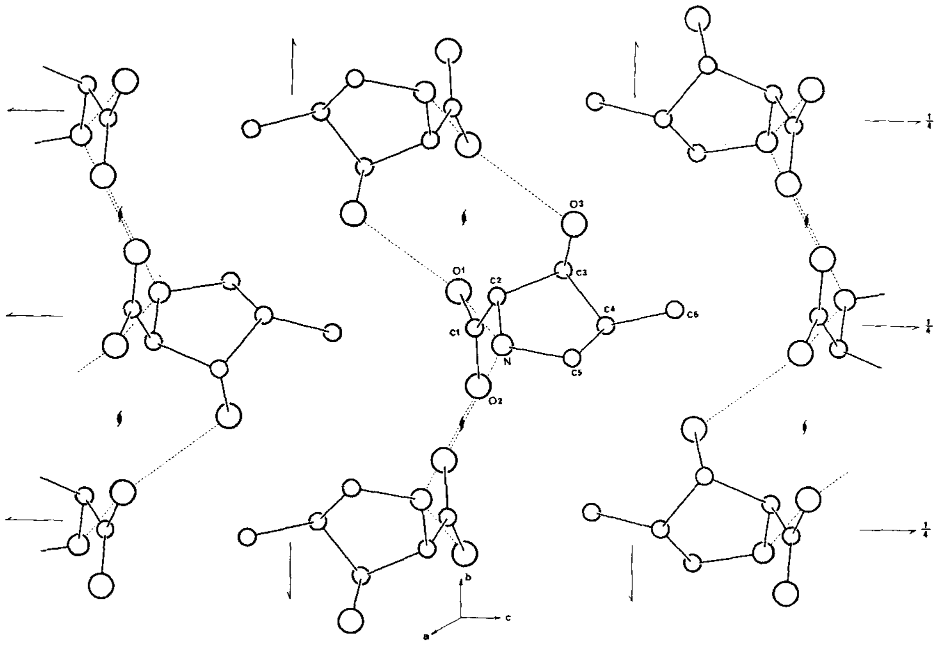

Fig. 3. *Molecular packing and hydrogen bond scheme*

The author wishes to acknowledge his indebtedness to Prof. *W. Keller-Schierlein* for providing the sample and to Mr. *Paul Seiler* and Dr. *Fritz Winkler* for their help at various stages of this work. He also thanks Prof. *W. Keller* and Prof. *J. D. Dunitz* for providing laboratory facilities and the ETH Zürich for the award of a research Fellowship.

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269. Extraction liquide-liquide par des esters alkylphosphoriques. III[1]) Séparation de la paire yttrium – gadolinium par l'acide di-(2-éthylhexyl)phosphorique[2])

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(19. IX. 74)

Summary. The separation of yttrium and gadolinium by solvent extraction with di-(2-ethylhexyl)phosphoric acid (H(DEHP)) from solutions in HNO_3 has been studied. The extraction takes place by the same mechanism for both elements. The extraction by the ion-exchange reaction is more selective than the extraction by the solvatation reaction. Methyl isobutyl ketone, compared to kerosene as diluent for H(DEHP), reduces more the extraction of Y than that of Gd, without affecting the mechanisms.

Introduction. – Dans un mémoire précédent [3], nous avons communiqué les résultats des extractions de $Y(NO_3)_3$ et de $Y(ClO_4)_3$ par l'acide di-(2-éthylhexyl)-phosphorique (H(DEHP)). Dans le présent travail nous démontrons les possibilités d'appliquer cet extractant à la séparation de la paire yttrium-gadolinium, les deux constituants principaux de la gadolinite.

1. Système $Gd^{3+}-H^+-NO_3^--H_2O-H(DEHP)$/kérosène. – Nos résultats sont compilés dans le tableau 1, et les essais Nos 1 à 6 sont transposés dans la fig. 1a. Le comportement de $Gd(NO_3)_3$ est analogue à celui de $Y(NO_3)_3$ [3]. Cependant, les coefficients de partage sont considérablement plus faibles; le domaine d'acidité où il y a extraction par échange d'ions uniquement est plus restreint ($[HNO_3] < 2$ M au lieu de <3 M) et le minimum d'extraction se situe à une acidité plus faible ($[HNO_3] = 5$–6 M au lieu de 7–8 M dans le cas de $Y(NO_3)_3$).

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[1]) Communication précédente: [1].
[2]) Extrait de la thèse de *W. Hirsbrunner* [2].