CRYSTAL STRUCTURES OF DIHYDRO-α-ERGOKRYPTINE AND DIHYDRO-β-ERGOKRYPTINE MESYLATES+

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Dedicated to Professor Josef Loub on the occasion of his 70th birthday.

The absolute crystal structures of two related ergot alkaloids dihydro-α-ergokryptine mesylate (**1**) monohydrate ethanol solvate and dihydro-β-ergokryptine mesylate (2) monohydrate methanol solvate have been determined by X-ray diffraction and compared with the published structures of dihydroergocristine mesylate (**3**) monohydrate and dihydroergotamine mesylate (**4**) monohydrate.

Key words: Ergot alkaloids; Dihydro-α-ergokryptine; Dihydro-β-ergokryptine; X-Ray structure.

Ergot alkaloids represent a series of pharmacologically active metabolites of parasitic fungi of the genus Claviceps which have been known since the Middle Ages. These alkaloids are used in their natural or chemically modified forms as important drugs[2]. Although they are very similar, their binding to various neuroreceptors is in some cases remarkably different for individual alkaloids[3,4], what is also reflected in their different therapeutic use. Whereas ergotamine and dihydroergotamine are used mainly in the treatment of migraine[5], dihydroergopeptines of the ergotoxine family are used for the treatment of complex of elderly diseases[6–9]. There is a general tendency in the medicinal use of ergot alkaloids to switch from the original

+ In this 21st paper on structure and polymorphism of ergot derivatives we report a comparison of four structurally related dihydroergopeptines. For the preceding paper of the series see ref.[1]

use of the dihydroergotoxine mixture (comprising four alkaloids: dihydroergocristine, dihydro-α-ergokryptine, dihydro-β-ergokryptine, and dihydroergocornine) to the use of single components in order to achieve better-defined mode of action. Increased attention dedicated to the treatment of elderly patients, mechanism of action of individual alkaloids and some of their novel pharmacological activities[10–12], prompted us to study in detail their conformation and structural parameters.

EXPERIMENTAL

Preparation of Crystals

Dihydro-α-ergokryptine mesylate (**1**) and dihydro-β-ergokryptine mesylate (**2**) are products of Galena Co. (Czech Republic). Compound **1** (100 mg) was dissolved in ethanol (3 ml) and pentyl acetate (7 ml) was added with stirring; crystals of **1** ethanol solvate monohydrate were obtained by partial evaporation of ethanol within one week. Compound **2** (100 mg) was dissolved in the mixture of methanol (1.25 ml) and water (50 μl) at 50 °C and ethyl acetate (15 ml) was added to this solution with stirring; the mother liquor was allowed to cool in room temperature; crystals of **2** methanol solvate monohydrate were formed overnight. Since both **1** and **2** prone to desolvation, the single crystals were adjusted in capillaries with small amount of mother liquor.

Crystal Structure Determination

Data collection and refinement parameters are listed in Table I, final positional and thermal parameters are deposed in CSD. International tables for X-ray crystallography[13] and following programs were used for calculations: SDP (ref.[14]), CRYSTALS (ref.[15]), PARST (ref.[16]), SHELXS86 (ref.[17]), XPMA, ZORTEP (ref.[18]). For the peptide-like numbering of atoms, see Fig. 1.

*Crystal data for **1** ethanol solvate monohydrate*: M_r = 731.86, space group $P2_12_12_1$ (No. 19), a = 11.756(1), b = 15.573(1), c = 21.009(4) Å, V = 3 846.3(8) Å^3, λ = 1.54184 Å, D_c = 1.274 g cm^{-3}, Z = 4, F(000) = 1 584, colourless blocks, crystal fragment of dimensions 0.56 × 0.56 × 0.75 mm, μ(CuKα) = 12.54 cm^{-1}. The structure was solved by direct methods and anisotropically refined by full-matrix least-squares. Hydrogen atoms were located (not refined) from a difference map and from expected geometry. Absolute configuration was proved with a value of the Flack's enantiopole parameter[19] of 0.04(2).

*Crystal data for **2** methanol solvate monohydrate*: M_r = 722.872, space group $P2_12_12_1$ (No. 19), a = 11.730(5), b = 15.490(1), c = 20.520(2) Å, V = 3 728(2) Å^3, D_c = 1.2878 g cm^{-3}, Z = 4, F(000) = 1 548, μ(MoKα) = 12.837 cm^{-1}. The structure was solved by direct methods and anisotropically refined by full-matrix least-squares. The two valine methyl groups were found dislocated in three positions of 2/3 occupancy level. Hydrogen atoms were localized from Δρ map and the expected geometry. The positions of the H-atoms were refined, except for H151, H152, H153, H221, H271, H272, H273, H281, H282, H283, H321, H322, H323, H501, H502, H503, H901, H902, H903, that were linked to the attached atoms. The value of 0.00(2) was evaluated for the Flack's enantiopole parameter[19]. The presence of solvent molecules was revealed from the Δρ map, only methanol hydrogen was not localized.

RESULTS AND DISCUSSION

Dihydro-α-ergokryptine and dihydro-β-ergokryptine mesylates crystallize from either pure ethanol or methanol or from their mixtures with various aliphatic esters with one molecule of water and one molecule of alcohol. However, these solvates are very unstable and even a decrease in the alcohol content in the mother liquor causes decomposition of crystals. Hence, they were adjusted in capillaries for the diffraction studies. A comparison of structural parameters of **1** and **2** indicates that compounds are roughly

TABLE I
Data collection and refinement parameters

Parameters	**1**	**2**
Crystal dimensions, mm	0.56 × 0.56 × 0.75	0.25 × 0.25 × 0.44
Diffractometer and radiation used, Å	Enraf–Nonius CAD4, CuKα, λ - 1.54184	
Scan technique	ω/2θ	
Temperature	293 K	
No. and θ range of reflections for lattice parameter refinement, °	20; 38–40	
Range of *h*, *k*, and *l*	0→14, 0→18, –25→25	0→12, 0→16, –21→21
Standard reflections monitored in the interval, min; intensity fluctuation, %	60; 3.27	60; 1.7
Total number of refections measured; 2θ range, °	6 919; 4–140	4 994; 4–110
No. of observed reflections	6 099	4 561
Criterion for observed reflections	$I \geq 1.96\sigma(I)$	
Function minimized	$w(\|F_o\| - \|F_c\|)^2$	
Weighting scheme	Chebychev polynomial (ref.[32])	
Parameters refined	462	578
Value of *R*, *wR*, and *S*	0.0647, 0.0749, and 1.0736	0.0497, 0.0421, and 1.0720
Ratio of the maximum least-squares shift to e.s.d. in the lst cycle	0.077	0.004
Maximum and minimum heights in final Δρ map, e $Å^{-3}$	0.68, –0.87	0.29, –0.42

isostructural. The absolute configuration was resolved as: C4 (*R*), C6 (*R*), C8 (*R*), C17 (*R*), C19 (*S*), C24 (*S*), C25 (*S*) for both compounds (Figs 1 and 2). In addition, the *S*-configuration of the C29 atom confirms the presence of L-isoleucine in **2**.

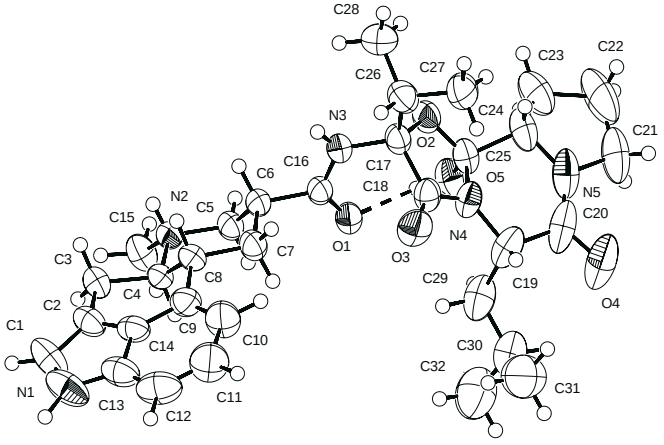


FIG. 1

ORTEP view of dihydro-α-ergokryptine cation, showing the numbering scheme. Thermal ellipsoids are drawn at 50% probability

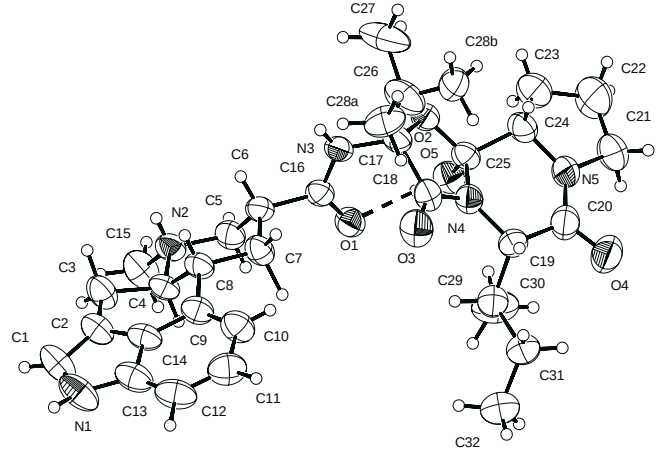


FIG. 2

ORTEP view of dihydro-β-ergokryptine cation, showing the numbering scheme. Thermal ellipsoids are drawn at 50% probability

As expected, the ergoline rings A, B and the cyclole ring E are nearly planar. Conformations of the flexible rings and their comparison with that published in dihydroergocristine mesylate (**3**) monohydrate[20] and dihydroergotamine mesylate (**4**) monohydrate[21] are shown in Table II. If there was no "pure" conformation observed, two assignments are used in sequence respecting the "closer" one. Protonization of the N2 atom does not affect a regular chair conformation of the ring D. However, we have inspected parameters, that have been reported as crucial ones for ergot alkaloid activity on 5-HT_{1A} receptors[21–23] (Table III). Surprisingly, the values of *h* (distance of N2 from the plane defined by the ring A) obtained for **1** ethanole solvate monohydrate and **2** methanole solvate monohydrate species significantly differ from known structures of ergot alkaloids. It is worth to mention that such values were observed only in N-methylbenzergolinium mandelate[24] (**5**, (–)-*trans*-4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-*ab*]phenanthridinium (–)-maleate) and nicergoline[25,26] (**6a**, **6b**, (10α-methoxy-1,6-dimethylergolin-8β-yl)methyl 5-bromonicotinoate) (Table III), which is also used for the treatment of some elderly diseases. Generally, the DH-ergopeptines are represented with remarkably higher *h* values in comparison with natural ergopeptines. Hence, the different therapeutic activity of the DH-ergopeptines may be related with that parameter not only at 5-HT_{1A}, but also at some other receptors, *e.g.* $GABA_A$ (ref.[27]).

Close examination of "puckering parameters" reveals, that not the quality of conformation (shifted E_3-$^4H_3$), but the value of the amplitude parameter of the ring C is responsible for such a deviation. The present structures extend current set of ergot alkaloid structures consisting of ergoline skeleton, where variability of the ring C is remarkably higher than that observed

TABLE II
Conformations of the various rings[33] in **1** ethanol solvate monohydrate, **2** methanol solvate monohydrate, **3** monohydrate, and **4** monohydrate.

Compound	C[a]	D[b]	F[c]	G[d]
1	E_3–$^4H_3$	$^4C_1$	^6E	^4E–$^4T_5$
2	E_3–$^4H_3$	$^4C_1$	^6E–$^6H_5$	$^4T_5$
3	E_3	$^4C_1$	^6E	^4E–$^4T_5$
4	E_3	$^4C_1$	^6E	$^4T_5$

[a] Ring C: C2,C3,C4,C8,C9,C14. [b] Ring D: N2,C4,C8,C7,C6,C5. [c] Ring F: N4,C19,C20,N5,C24,C25. [d] Ring G: N5,C21,C22,C23,C24.

for the ring D. Both leucine and isoleucine in the tripeptide moieties exhibit usual *gauche I* conformation [Leu: ψ_3(N4–C19–C20–N5) = –9.1(6)°, χ_3^{11}(N4–C19–C29–C30) = –173.2(4)°, χ_3^{21}(C19–C29–C30–C31) = 55.1(6)°, χ_3^{22}(C19–C29–C30–C32) = 178.8(4)°; Ile: ψ_3(N4–C19–C20–N5) = –3.3(4)°, χ_3^{11}(N4–C19–C29–C30) = –149.0(3)°, χ_3^{12}(N4–C19–C29–C30) = 84.0(4)°, χ_3^{21}(C19–C29–C31–C32) = 171.7(3)°].

The hydrogen bond network is virtually the same for **1** ethanol solvate monohydrate and **2** methanol solvate monohydrate (Fig. 3; Table IV). There is an "obligatory" O5–H501···O1 intramolecular hydrogen bond[29–30]. The mesylate anion is bound to the ergopeptine moiety through the N2–H621···O6 link, water molecule through the O100–H1001···O82 contact and ethanol–methanol solvent with the N1–H611···O91. The distortion of the N1–H611···O91 angle in **1** ethanol solvate monohydrate is probably due to inaccurate H611 position. The bridge to another ergopeptine molecule is then formed with the O100–H1002···O4 ($-x + 1/2$, $-y + 1$, $z - 1/2$) bond.

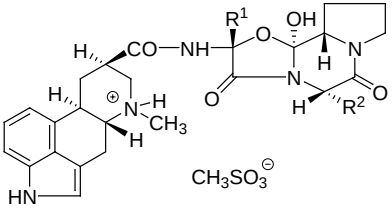
TABLE III
Parameters of 5-HT_{1A} pharmacophore model

Compound	*d*, Å[a]	*h*, Å[b]
1 Ethanol solvate monohydrate	5.23	0.96
2 Methanol solvate monohydrate	5.22	0.98
3 Monohydrate	5.25	0.74
4 Monohydrate	5.24	0.70
5[c]	5.20, 5.18	0.93, 1.05
6[d]	5.21	0.83
6[e]	5.21	0.81
7[f] Acetone solvate	5.17	0.72
8[g] Monohydrate	5.22	0.50
9[h]Methanol solvate	5.23	0.57
10[i]	5.19	0.19

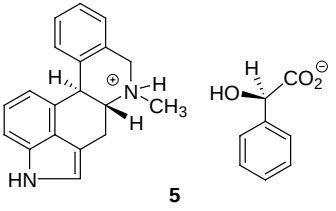
[a] Distance between the center of the ring A and the N2 atom. [b] Distance of the N2 atom from the plane defined by the ring A. [c] Two independent molecules in the structure of **5**. [d] Nicergoline, low-melting orthorhombic form II. [e] Nicergoline, high-melting triclinic form I. [f] Ergocristine (**7**) acetone solvate[30]. [g] Ergogaline (**8**) monohydrate[31]. [h] Ergotamine tartrate (**9**) methanole solvate[34]. [i] Bromocriptine mesylate[35] (**10**).

Surprisingly, in contrast to both **3** monohydrate and **4** monohydrate hydrogen networks, no N1–H611···O3′ contact was found. Steric proximity of an alcohol and the alkyl chains of leucine or isoleucine in the structures of **1** ethanol solvate monohydrate and **2** methanol solvate monohydrate indicates that alcohols fill the free space occupied by more steric demanding benzyl group in the structures of **3** monohydrate and **4** monohydrate. This fact seems likely to explain the differences in solvation between dihydroergokryptine mesylates monohydrates alcohol solvates and **3** and **4** monohydrates.

In contrast to natural ergopeptines, where the overall conformation brings the side chain of the third amino acid moiety (Leu, Ile, Phe, see Fig. 1) to close proximity of lysergic acid moiety (see, *e.g.*, data for ergogaline[31]), such direct contact is absent in dihydroergopeptines. Since this part of molecule



1, $R^1 = CH(CH_3)_2$, $R^2 = CH_2CH(CH_3)_2$
2, $R^1 = CH(CH_3)_2$, $R^2 = -C(H)(CH_3)-CH_2CH_3$
3, $R^1 = CH(CH_3)_2$, $R^2 = CH_2C_6H_5$
4, $R^1 = CH_3$, $R^2 = CH_2C_6H_5$



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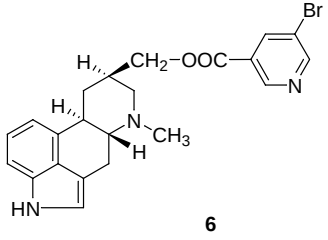

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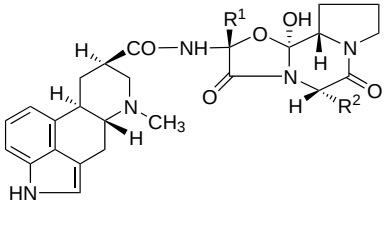
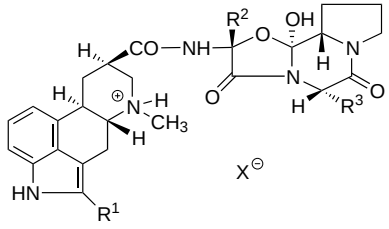


7, $R^1 = CH(CH_3)_2$, $R^2 = CH_2C_6H_5$
8, $R^1 = CH(CH_3)_2$, $R^2 = CH_2-C(H)(CH_3)-CH_2CH_3$



9, $R^1 = H$, $R^2 = CH_3$, $R^3 = CH_2C_6H_5$, X = L-tartrate
10, $R^1 = Br$, $R^2 = CH(CH_3)_2$, $R^3 = CH_2CH(CH_3)_2$, X = mesylate

TABLE IV
Hydrogen bonds in **1** ethanol solvate monohydrate and **2** methanol solvate monohydrate

D–H···A	Symmetry code	D···A, Å		D–H···A, °	
		1	**2**	**1**	**2**
O5–H501···O1	*x*, *y*, *z*	2.829(3)	2.845(3)	146	167(4)
N1–H611···O91	*x*, *y*, *z*	3.037(7)	2.937(7)	127[a]	159(5)
N2–H621···O81	*x*, *y*, *z*	2.812(4)	2.866(4)	169	175(3)
N3–H631···O100	*x*, *y*, *z*	2.958(4)	2.901(4)	174	161(3)
O100–H1001···O82	*x*, *y*, *z*	2.865(5)	2.862(4)	164	177(6)
O100–H1002···O4	–*x*+1/2, –*y*+1, *z*–1/2	2.862(5)	2.812(5)	173	168(4)

[a] Slightly shifted hydrogen position.

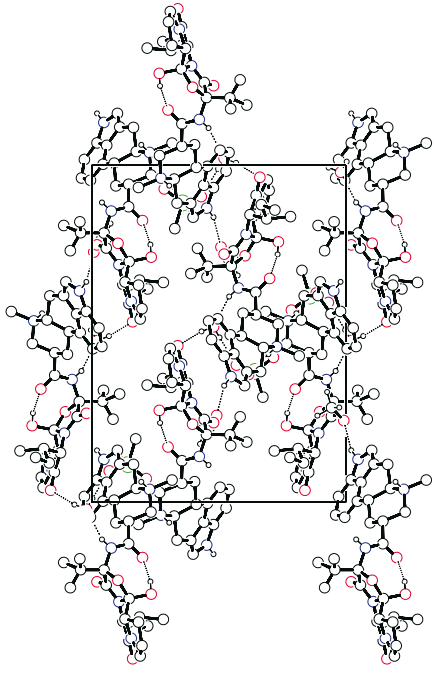


FIG. 3
Packing scheme of dihydro-β-ergokryptine mesylate (**2**) monohydrate ethanol solvate; dashed lines indicate hydrogen bonds (a view in 0 → *x* direction)

is considered to be responsible for binding to the receptor, the differences in the steric hindrance seem likely to contribute to the difference between the pharmacological effect of ergopeptines and dihydroergopeptines. However, a comparison of molecular shape of **1**, **2**, **3**, and **4** reveals, that their inter-individual differences are rather subtle, and thus they cannot satisfactorily explain the differences in pharmacological activity within this series.

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REFERENCES

1. Cvak L., Stuchlík J., Schreiberová M., Sedmera P., Havlíček V., Flieger M., Čejka J., Kratochvíl B., Jegorov A.: *Collect. Czech. Chem. Commun*, in press.
2. Brossi A. (Ed.): *The Alkaloids, Chemistry and Pharmacology*, Vol. 38, p. 1. Academic Press, Inc., San Diego 1990.
3. Korneyev A. Y., Cincotta A. H.: *Life Sci.* **1996**, *58*, 241.
4. Eich E, Pertz H.: *Pharmazie* **1994**, *49*, 867.
5. Siberstein S. D., Young W. B.: *Neurology* **1995**, *45*, 577.
6. Scarzella L., Bono G., Bergamasco B.: *Int. J. Clin. Pharm. Res.* **1992**, *12*, 37.
7. Franciosi A., Zavattini G.: *Curr. Ther. Res.* **1994**, *55*, 1391.
8. Ammon R., Sharma R., Gambert S. R., Gupta K. L.: *Age* **1995**, *18*, 5.
9. Cucinotta D., De Leo D., Frattola L., Trabucchi M., Parnetti L: *Arch. Gerontol. Geriatr.* **1996**, *22*, 169.
10. Favit A., Sortino M. A., Aleppo G., Scapagnini U., Canonico P. L.: *Pharmacol. Toxicol.* **1993**, *73*, 224.
11. Coppi G.: *J. Neural Transm., Suppl.* **1995**, *45*, 307.
12. Li A., Sahm U., Artmann G. M.: *Clin. Hemorheol.* **1995**, *15*, 133.
13. *International Tables for X-ray Crystallography,* Vol. IV. Kynoch Press, Birmingham 1974.
14. Frenz, B. A. and Associates Inc. SDP: *Structure Determination Package*, Enraf–Nonius, Delft 1985.
15. Watkin D. J., Carruthers R. J., Betteridge P.: *CRYSTALS*, Issue 10. Chemical Crystallography Laboratory, Oxford 1998.
16. Nardelli M.: *PARST, a System of Computer Routines for Calculating Molecular Parameters from Results of Crystal Structure Analysis*. University of Parma, Parma 1998.
17. Sheldrick G. M.: *SHELXS86, Program for Crystal Structure Solution*. University of Göttingen, Göttingen 1986.
18. Zsolnai L., Huttner G.: *XPMA, ZORTEP*. University of Heidelberg, Heidelberg 1994.
19. Flack H. D.: *Acta Crystallogr., Sect. A: Fundam. Crystallogr.:* **1983**, *39*, 876.
20. Čejka J., Ondráček J., Hušák M., Kratochvíl B., Jegorov A., Stuchlík J.: *Collect. Czech. Chem. Commun.* **1995**, *60*, 1333.
21. Kidrič J., Kocjan D., Hadži D.: *Experientia* **1986**, *42*, 327.
22. Hibert M. F., Gittos M. F., Middlemiss D. N., Mir A. K., Fozzard J. R.: *J. Med. Chem.* **1988**, *31*, 1087.

23. Bolis G., Pillan A., Mantegani S., Brambilla E., Dolmella A.: *J. Mol. Model.* **1995**, *1*, 188.
24. Seiler M.P., Floersheim P., Markstein R., Widmer A.: *J. Med. Chem.* **1993**, *36*, 977.
25. Hušák M., Kratochvíl B., Ondráček J., Maixner J., Jegorov A., Stuchlík J.: *Z. Kristallogr.* **1994**, *209*, 260.
26. Hušák M., Had J., Kratochvíl B., Cvak L., Stuchlík J., Jegorov A.: *Collect. Czech. Chem. Commun.* **1994**, *59*, 1624.
27. Tvrdeić A., Peričić D.: *Eur. J. Pharmacol.* **1991**, *202*, 109.
28. Hebert H.: *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1979**, *35*, 2978.
29. Weber H. P. in: *Ergot Compounds and Brain Function* (M. Goldstein, A. Liberman, D. B. Calne and M. O. Thorner, Eds), p. 25. Raven Press, New York 1980.
30. Pakhomova S., Ondráček J., Hušák M., Kratochvíl B., Jegorov A., Sedmera P., Havlíček V.: *Z. Kristallogr.* **1997**, *212*, 593.
31. Cvak L., Jegorov A., Sedmera P., Havlíček V. Ondráček J., Hušák M., Pakhomova S., Kratochvíl B., Granzin J.: *J. Chem. Soc., Perkin. Trans 2*, **1994**, 1861.
32. Carruthers J. R., Watkin D. J.: *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1979**, *35*, 698.
33. Cremer D., Pople J. A.: *J. Am. Chem. Soc.* **1975**, *97*, 1354.
34. Pakhomova S., Ondráček J., Kratochvíl B., Jegorov A.: *Z. Kristallogr.* **1996**, *211*, 555.
35. Hušák M., Kratochvíl B., Jegorov A., Cvak L.: *Z. Kristallogr.* **1997**, *212*, 39.