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FLUOROIMINES FROM THE REACTION OF FLUOROAMINO ACIDS OR FLUOROKETO ACIDS WITH THE ALDEHYDE OR AMINE FORM OF VITAMIN B_6: PART III[+]. INFLUENCE OF FLUORINE ON THE FORMATION AND THE REACTIVITY OF FLUOROIMINES DERIVED FROM β-FLUOROASPARTATES OR β-FLUOROOXALOACETATE

M.C. SALON, S. HAMMAN and C.G. BEGUIN*

Université de Grenoble I, CERMO, BP 68, F 38402 St Martin d'Hères (France)

SUMMARY

The introduction of fluorine at the β-position in aspartate and in oxaloacetate, the typical amino acid-keto acid couple in transamination, induces large effects in their reaction with respectively pyridoxal 5'-phosphate (PLP) (1) and pyridoxamine 5'-phosphate (PMP) (4). The formation of imine intermediates through reaction of 1 with erythro or threo-β-fluoroaspartate (2e and 2t) and through reaction of 4 with β-fluorooxaloacetate 5 is highly favored in comparison with that of non-fluoro compounds. The stereoisomers of the imines are unambiguously determined using a reduction reaction. The evolution of these intermediates shows that no transamination is observed and that a dehydrofluorination occurs suggesting that the β-fluoro moiety in the intermediate turns into a good leaving group, changing the model reaction of transamination on α carbon through the aldimine-ketimine tautomerization to an elimination on β carbon.

INTRODUCTION

Transaminases catalyse the conversion of amino acids to α-keto acids, involving the aldehyde form of the coenzyme, pyridoxal 5'-phosphate (PLP) 1, and its amine form, pyridoxamine 5'-phosphate (PMP) 4 [2-4]. One of the most studied amino acid-keto acid couple is aspartate-oxaloacetate. Model studies [2,4] have shown that the slow step in this reaction is the prototropic shift in the aldimine $\rightleftharpoons$ ketimine tautomerization

[+] Part II: reference [1].

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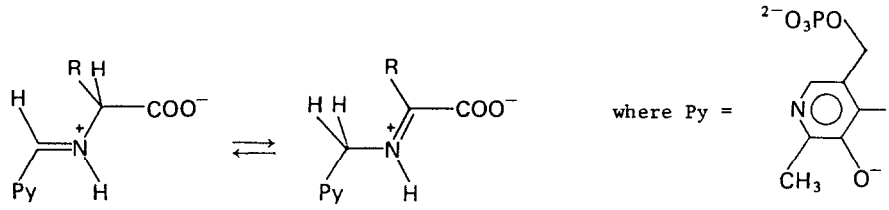


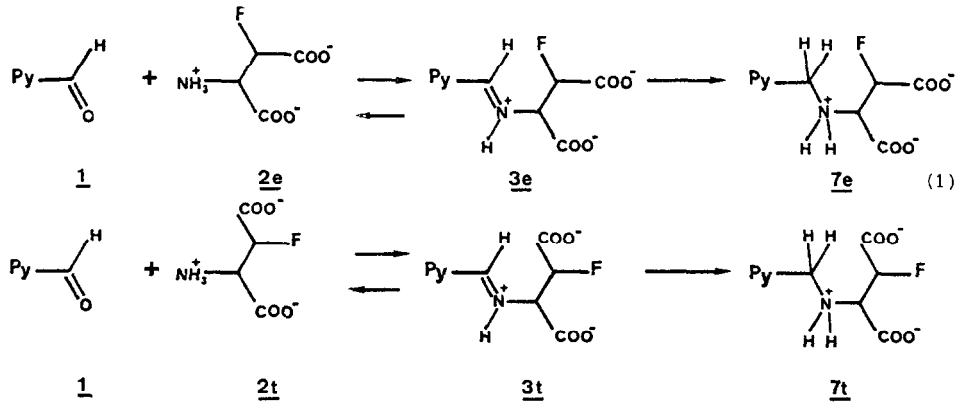
The use of fluorinated substrate analogues [5-6] as effectors (competitive inhibitors [6], suicide substrates [7-9] or physical probes [10]) of transaminases or related enzymes has received considerable attention over the last few years. Introduction of fluorine into the two most important interconverting substrates in transamination has been undertaken: β-fluoroaspartates (β-F Asp) 2 [with its two diastereoisomers (αR^*, βR^*)-β-fluoroaspartate or erythro-β-fluoroaspartate 2e and (αR^*, βS^*)-β-fluoroaspartate or threo-β-fluoroaspartate 2t] and β-fluorooxaloacetate 5. The ^{19}F NMR parameters of these compounds and of the reaction products 1 with 2 and 4 with 5 and the corresponding reduction products have already been published for unambiguous assignment of the stereoisomers [1].

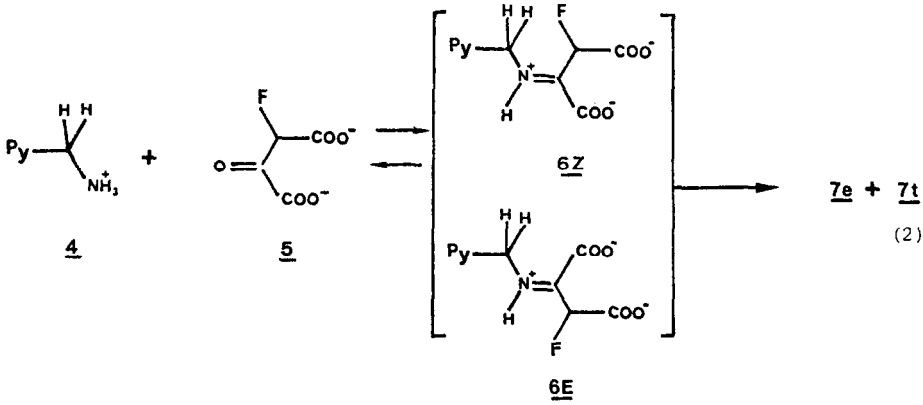
The aim of this paper is to describe the effect of fluorine in aspartate and oxaloacetate on the reactivity of these substrates on PLP and PMP respectively. Specifically we sought to determine the influence of fluorine on (i) the formation of the imine intermediates and (ii) the evolution of these intermediates through reactions on α-carbons (transamination, racemisation or decarboxylation) or on β-carbons (elimination).

RESULTS

The formation of imines (aldimine 3 by reaction of 2 with 1 and ketimines 6 from 4 with 5) was studied in aqueous solution (in general, in D_2O for easy locking on the deuterium signal in NMR experiments) over the pD range 1-12. For unambiguous identification of 3 and 6, reduction by sodium borohydride was undertaken. The isolation of a unique dihydro-N-pyridoxylidene 5'-phosphate β-fluoroaspartate 7, from the two imines 3 and 6 was clear evidence of isomerism of 3 and 6.

Figure 1 shows all the different reaction products taking account of the assignment of the stereoisomers [1] and of the preferential conformations around the C_α-C_β bond.





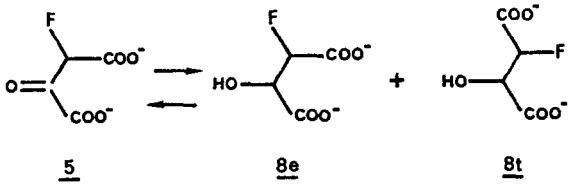


Fig. 1. Reaction products.

Reaction (1) of fluoroaspartates with PLP

2e was observed in solution over the entire pD range. 2t could not be observed at pD > 6.5. When 2e (2t) was mixed with 1, a rapid reaction occurred and gave 3e (3t). The observed aldimine has the same configuration as the starting amino acid and the stereochemistry around the imine double bond (with the protonated imine nitrogen) is likely E [1]. The reaction was rapid on the NMR time scale. For very acidic media, the formation of 3 was negligible. The aldimine and its amino acid are equilibrated with 1 with a pD profile with a maximum at pD = 5.5 for 2t. The apparent equilibrium constant for this pD is about 20. This value is an approximate one since a drift occurs versus time. This drift, relatively fast in basic media, is related with the appearance of fluoride ion in the medium, through dehydrofluorination. The addition of the cation Al^{3+} (which increases the aldimine amount for non-fluorinated amino acids [12-13]) only increases the rate of dehydrofluorination.

The reduction of 3 with sodium borohydride gave the corresponding dihydro-N-pyridoxylidene 5'-phosphate β-fluoroaspartate 7 with the same stereochemistry as the starting compound. This trapping of 3 with sodium borohydride shifted the equilibrium reaction of aldimine formation since no more fluoro-amino acid was observed whatever was the reaction time.

Reaction (2) of monofluorooxaloacetate with PMP

5 was observed in solution over the entire pD range. 5, alone in solution, underwent incorporation of deuterium at the β-position, with a rate depending on pD, in relation with its enolization (see experimental part).

When 5 was mixed with 4, for pD > 8.5, a slow reaction occurred and gave 6 (α-pyridoximino-5'-phosphate β-fluoro-oxaloacetate) with two stereoisomers (E and Z around the imine double bond with the protonated nitrogen). An apparent equilibrium constant for reaction (2) could be obtained: $K = [6E + 6Z]/[4][5]$. Its variation as a function of pD and with initially containing 0.1:0.1 M; 0.1:0.2 M; 0.2:0.1 M concentrations of 5 and 4 respectively is shown in Table 1

Table 1. Variation of apparent equilibrium constant in reaction (2) versus pD from solutions initially containing 5 and 4

pD	9.0	9.5	10.0	10.4	11.0	11.5	11.75
K [a]	6.5	12	20	60	23	12	7

[a] reference concentrations expressed in M.

The reversibility of this reaction was tested using wide pD variations. The apparent equilibrium constant value was reached after almost four hours, then this value was slightly drifting, as a slow decrease of the signals due to 6E and 6Z was observed, accompanied by a slow increase of the fluorine anion signal.

For initial solutions containing 0.1:0.2 M of 5 and 4, dehydrofluorination was no longer negligible and the apparent equilibrium constant was strongly drifting. Addition of aluminium cations (0.05 M) produced dehydrofluorination (F^- signal) at a rate from 6Z larger than from 6E.

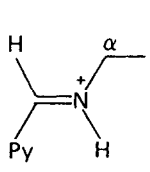
6E was always more abundant than 6Z (relative molar ratio 0.66:0.33 at pD 10, for instance).

The reduction of 6 with sodium borohydride gave the two diastereoisomers of dihydro-N-pyridoxylidene 5'-phosphate β-fluoroaspartate 7e and 7t, with deuterium incorporated at β-position. For unambiguous comparison with the compounds obtained from 3, reaction (2) was undertaken in the mixture H_2O:D_2O (90:10): 7e and 7t with about 10% of the corresponding β-deuterated compounds were obtained. The compounds 7e and 7t obtained from reduction of 3 or 6 were identical. As 6E and 6Z were obtained in equilibrium with 5, the reduction of the equilibrated mixture by sodium borohydride gave not only 7e and 7t, but also the two diastereoisomers of fluoromalate (R^*R^* or erythro 8e and R^*S^* or threo 8t) [14-15].

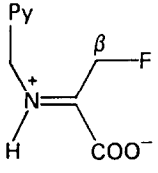
INTERPRETATION

Stereochemistry of imine formation

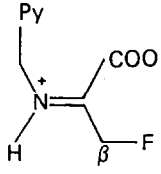
Imine formation occurs after dehydration of the corresponding carbinolamine. The carbinolamines result from nucleophilic attack of the NH_2 group of 2 or 4 on the carbonyl group of 1 or 5 respectively. Examination of the preferential conformations along the CN bond of carbinolamines and the disubstituted or trisubstituted character of the three protonated imines 3, 6E and 6Z



3



6E



6z

suggests that no isomerisation around the imine bond occurs in 3 and that the E isomer is more stable than Z in 6. β-Fluorine is a probe for easy distinction by ^{19}F NMR of the two stereoisomers of 6 (large chemical shift difference and occurrence of $^3J_{FN}$ or $^3J_{FD}$ on the observed $^2J_{FD}$ coupling constants) [1].

Stereochemistry and relative rates of reduction of 5 and 6

The stereoisomers of 7e, 7t, 8e and 8t were unambiguously assigned previously [1]. For discussion of the asymmetric induction during reduction by sodium borohydride, we used Felkin's model [16] on the unsaturated carbon >C=Z (Z = O for 5 and $=\overset{+}{N}<$ for 6): the transition state during the approach of the reducing agent is stabilized if the incipient bond (between the nucleophile and the unsaturated carbon) is antiperiplanar to the largest group on C_β (COO^-). Figure 2 shows that the major attack, for both cases (=O and $=\overset{+}{N}<$)

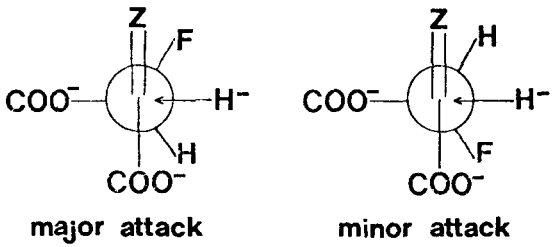


Fig. 2. Comparison of attack levels.

gives the erythro compound, probably to a greater extent for 5 (as Z is larger in 6): indeed the 8e:8t molar ratio is about 0.79:0.21, and 7e:7t is 0.71:0.29

The significantly smaller molar ratio for reduction products 7:8 (0.62:0.38) in comparison with that of the starting compounds 6:5 (0.72:0.28) can be interpreted by a greater reactivity of the carbonyl group of 5 than that of the sterically larger imine group of 6. Another interpretation is that the introduction of the reducing agent, sodium borohydride, rapidly increases the pD and therefore decreases the amount of 6 in reaction (2).

Rate and equilibrium of imine formation

Imine (3e or 3t) formation is fast on the NMR time scale, as it is in the case of aspartate [13]. Nevertheless, at moderate pH, the formation of imine is relatively more important than with aspartate. This can be related to the electron-withdrawing effect of fluorine on basicity of the NH_2 groups of 2e or 2t: the $p\overset{+}{K}NH_3$ values of aspartate (Asp), 2e, and 2t are respectively: 9.7, 7.4, and 6 < pK(2t) < 7.4. It has been established [13] that the equilibrium constant of formation of imine from amino acid and 1 has a pH-profile with a maximum at pH = 1 ($pK_{P1} + pK_{D1}$) where pK_{P1} is the pK of the last acidity of 1

(8.1) and pK_{D1} is the pK of the last acidity of the amino acid. A decrease of pK from Asp to 2 leads to a decrease of the maximum of the pH profile. So at moderate pH, the apparent equilibrium constant of imine 3 seems higher than for Asp.

Imine formation is favorable for a pH profile with a maximum at pD = 10.4 (pH = 10), probably related with the nitrogen pK of 4 (10.5) [17] and with the pK of imine nitrogen of 6 also in this area [13]. The apparent equilibrium constant of imine formation at its maximum is relatively high (60). No ketimine could be detected with oxaloacetate and 4 [13]. The maximum equilibrium constant of the reaction of pyruvate with pyridoxamine [18,19] or 4 [20] has a maximum of about 5 at pD = 10. A maximum value of 15 at pD = 10 was observed with para-fluorophenylpyruvate and 4 [21]. This relatively favorable imine formation can be related to the electron-withdrawing effect of fluorine on the carbonyl group. The higher reactivity of 5 in comparison with oxaloacetate was also observed by one of us [22] when studying the hemiketal formation of these carbonyl groups with ethyl alcohol. With 5, the hemiketal is completely and rapidly obtained (two stereoisomers), whilst with oxaloacetate under the same conditions an equilibrium is slowly established.

Competition of dehydrofluorination and transamination

Direct transamination of 2 to 5 (or the reverse reaction) with 1 (or 4) has not been observed. Dehydrofluorination always occurred, through 3 (completed dehydrofluorination with 3t at pD = 5.5 after 1.30 h) or through 6 (slow dehydrofluorination after 4 h at pD = 10.4). The reaction products are fluoride anion, ammonia and oxaloacetate. This α-keto-acid arises from the rapid hydrolysis of the ketimine tautomer of the eneamino acid initially obtained through dehydrofluorination. The fluoride ion was easily detected by ^{19}F NMR. Addition of a cation such as Al^{3+}, a catalyst for transamination in the model reaction [4], increases sharply the rates of dehydrofluorination of 3 or 6.

The prototropy from aldimine to ketimine is therefore an unfavorable process with these fluorinated substrates, competing with a more efficient process, dehydrofluorination. This last reaction was observed previously when 2 was tested as an inhibitor of aspartate transaminase [23]: a very rapid dehydrofluorination occurred suggesting that 2 was unstable and could not be isolated. This competitive reaction α-β elimination, versus transamination has been observed before as a secondary activity of transaminases (for example dehydrochlorination of β-chloroglutamate with aspartate transaminase [24] or dehydrase activity, dehydration of serine with serine dehydrase[25]).

Model reactions of this β-elimination (dehydration) competing with dealdolisation on β-hydroxyamino acid as substrates (threonine [26] or with phenylserine [27]) with Al^{3+} as a catalyst were also studied. The influence of stereochemistry is quite important as shown by the greater sensitivity of 2t compared with 2e. Unambiguous methods for stereochemical assignments of all the assumed intermediates are therefore necessary [1].

EXPERIMENTAL

Pyridoxal 5'-phosphate (1) and pyridoxamine 5'-phosphate (4) were purchased from Merck and used without further purification. β-Fluoroaspartate (2) was prepared according to known procedures: 2t from diethyl threo-β-hydroxyaspartate with sulfur tetrafluoride in liquid HF [28] and 3e from erythro-β-hydroxyaspartate by the same procedure or by diazotization of meso-diamino succinic acid in liquid hydrogen fluoride [29].

The stereochemistry is unambiguous [11,30]. β-Fluorooxaloacetate (5) was obtained by hydrolysis of diethyl fluorooxaloacetate, prepared by condensation of diethyl oxalate with ethyl fluoroacetate in ethanol and sodium ethoxide [31]. The hydrolysis was carried out in a mixture 2:1 (v/v) of acetic acid and 6N hydrochloric acid at room temperature over 3 days. Recrystallization from light petroleum gave white crystals (m.p. 86°) [1].

The solutions in D_2O were usually 0.1 M and were adjusted with NaOD or DCl in D_2O and measured on an Orion digital pH meter with an Ingold electrode (405/M3). The pD was obtained from the pH meter readings under standard conditions by adding 0.40 units [32]. For comparison of pK in H_2O and in D_2O, the equation $pK_{D_2O} = 1.018\ pK_{H_2O} + 0.43$ was used [33].

The reduction by sodium borohydride was carried out by adding a slight excess of solid sodium borohydride [34].

The ^1H noise decoupled ^{19}F NMR spectra were obtained on a Bruker WP100 spectrometer at 94.18 M Hz in the Fourier transform mode with the conditions already described [1]. ^{19}F chemical shift titration curve analysis gives two pH jumps for 2e (2.7 and 7.4) and also for 2t (1.1 and about 6.5). The high rate of dedhydrofluorination for 2t can be related with the large population of the conformer where F and H are antiperiplanar [11], a favoured conformation for trans-dehydrofluorination.

^{19}F chemical shift titration curve shows for 5 a high pH jump (11.2 ppm) corresponding to a pK value of 2.3 (for the two carboxylic acid functions). 5, even alone in solution, underwent incorporation of deuterium at this β-position, with a rate depending on pD, in relation with its enolization.

Without PMP, at pD < 3 no deuteration was observed; after a long time (15 h for instance at pD 3), decarboxylation occurred (fluoropyruvate signal); in the pD range 3-6.5, a non-deuterated and a slowly obtained deuterated fluorine NMR signal were observed (in a mixture H_2O:D_2O 90:10, at pH = 4, after two hours, 10% of the deuterated 5 was observed). In the pD range 6.4-8.5, most of 5 was deuterated. With 4, even at concentrations much less than the equimolecularity, whole deuteration occurred and the rate was higher than previously; at pD 1.3, t 1/2 for deuteration was two hours, at pD 5 t 1/2 was 19 mn.

Wide pD variations, to test the reversibility of reaction (2), were undertaken as follows: the equilibrium was reached at pD 9.8 for initial concentrations 0.2 M of 4 and 0.1 M of 5 (three signals 6E, 5, 6Z with relative molar ratio 0.48, 0.28, 0.24). The pD rapidly decreased to 3.4. The signal due to 5 was only observed with all the intensity. Then the pD was returned to 9.8 and the three signals reappeared.

The molar ratios for the products obtained from reduction of 5 alone or of the mixture 5 + 6 were obtained from the signal area as follows: the molar ratios for the four products 8e, 7e, 7t and 8t were respectively 0.31, 0.44, 0.18, 0.07; from the mixture 6E, 5, 6Z (0.48, 0.28, 0.24). The relative ratios of 7e:7t (0.71:0.29) and of 6E:6Z (0.66:0.34) are both larger than one. As expected, 8e:8t (79:21) when reduction occurs on 5 only is almost the same as when it occurs (81:19) on 5 in the mixture 5 + 6. The molar ratio for 6:5 (0.72:0.28) is significantly different from that for 7:8 (0.62:0.38), suggesting that the rate of reduction of 5 is longer than that of 6.

REFERENCES

1 M.C. Salon, S. Hamman and C.G. Béguin, Org. Magn. Reson., 21 (1983) 265.
2 C. Walsh, Enzymatic Reaction Mechanisms, W.H.F. Freeman, San Francisco, 1979, p. 777.
3 E.E. Snell and S.J. Di Mari, in P.D. Boyer (ed.), The Enzymes, Academic Press New York, 1970, 3rd ed., vol. II, p. 366.
4 A.E. Martell, in A. Meister (ed.), Advances in Enzymology, Wiley, New York, 1982, vol. 53, p. 163.
5 C. Walsh, in A. Meister (ed.), Advances in Enzymology, Wiley, New York, 1983, vol. 55, p. 197
6 R. Filler, Am. Chem. Soc. Symp. Ser., 28 (1976).
7 R.H. Abeles and A.L. Maycock, Acc. Chem. Res., 9 (1976) 313.
8 J. Kollonitsch, A. Patchett, S. Marburg, A. Maycock, L. Perkins, G. Doldouras, D. Duggan and S. Aster, Nature, 174 (1978) 406.

9 P. Bey, in N. Seiler, M.J. Jung and J. Koch-Weser (eds), Enzyme-activated Irreversible Inhibitors, Elsevier, Amsterdam, 1978, p. 27.

10 J.T. Gerig, in L.J. Beihmer and J. Reuben (eds), Biological Magnetic Resonance, Plenum Press, New York, 1978, vol. 1, p. 139.

11 S. Hamman, C.G. Béguin, C. Charlon and C. Luu-Duc, Org. Magn. Reson., 21 (1983) 361.

12 O.A. Gansow and R.H. Holm, J. Am. Chem. Soc., 91 (1969) 573.

13 E. Gout, Thèse, Grenoble (1983).

14 J.A. Goldstein, Y.F. Cheung, M.A. Marletta and C. Nalch, Biochemistry, 17 (1978) 5567.

15 R. Heck, H. Haas and J. Retey, FEBS Letters, 114 (1980) 287.

16 M. Cherest, H. Felkin and N. Prudent, Tetrahedron Lett., (1968) 220.

17 S.A. Hershey, PhD Thesis, Ohio University (1976).

18 B.E.C. Banks, A.A. Diamantis and C.A. Vernon, J. Chem. Soc., (1961) 4235.

19 O.A. Gansow and R.H. Holm, J. Am. Chem. Soc., 91 (1969) 5984.

20 W.L. Felty and D.L. Leussing, J. Inorg. Nucl. Chem., 36 (1974) 617.

21 A. Dahbi, Thèse Grenoble (1983).

22 M.C. Salon, unpublished results.

23 E. Kun, D.W. Fanshier and D.R. Grassetti, J. Biol. Chem., 235 (1960) 416.

24 E. Antonini, M. Brunori, P. Fasella, R. Khomutoo, J. Manning and E. Severin, Biochemistry, 9 (1970) 1211.

25 L. Davis and D. Metzler in P. Boyer (ed.) The Enzymes, Academic Press, New York, 7 (1972) 33.

26 J.A. Marcello, A.E. Martell and E.H. Abbott, J. Chem. Soc. Chem. Comm. (1975) 16.

27 K. Tatsumoto and A.C. Martell, J. Am. Chem. Soc., 100 (1978) 5549.

28 J. Kollontisch, S. Marburg and L.M. Perkins, J. Org. Chem., 44 (1979) 771.

29 K. Matsumoto, Y. Ozaki, T. Iwasaki, H. Horikawa and M. Miyoshi, Experientia 35 (1979) 850.

30 K. Goubitz, H. Schenk and C.H. Stam, Cryst. Struct. Comm., 10 (1981) 1029.

31 J. Blank and J. Mager, Experientia, 10 (1954) 77.

32 P.K. Glascoe and L.A. Long, J. Phys. Chem., 64 (1960) 188.

33 H.J.C. Yeh, K.L. Kirk, L.A. Cohen and J.S. Cohen, J. Chem. Soc. Perkin II, (1975) 928.

34 J. Butler, W. Alworth and N. Nugent, J. Am. Chem. Soc., 96 (1974) 1617.