THERMAL AND SPECTRAL CHARACTERIZATION OF ISOOROTIC AND 2-THIOISOOROTIC ACIDS AND THEIR AMMONIUM, SODIUM AND BARIUM SALTS

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(Received 12 March 1990)

ABSTRACT

The spectral behaviour of isoorotic and 2-thioisoorotic acids, as well as of their ammonium, sodium and barium salts, has been studied by IR and ^{1}H and ^{13}C NMR spectroscopies. Similarly, thermal studies have been carried out by means of TG, DTG and DSC techniques, including infrared spectra of intermediate species in the pyrolytic decomposition of compounds.

INTRODUCTION

Orotic acid is the only effective precursor in the synthesis of pyrimidine bases for nucleic acid formation in living systems [1,2]. In addition to this biological importance, this compound is widely used on a clinical scale in the treatment of some pathologies [3], depending for its therapeutic action on the biological activity of its complexes with biometals [4]. For these reasons, chemical [5–10] and structural [11–17] information about metal
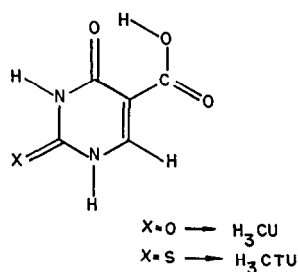
Scheme 1

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complexes of orotic acid and its derivatives has been the major aim of previously published research.

As an extension of our previous work on this subject [18–23], we report in this paper the spectral and thermal behaviour of two analogues of orotic acid, the isoorotic (H_3CU) and 2-thioisoorotic (H_3CTU) acids, as well as the corresponding ammonium, sodium and barium salts.

EXPERIMENTAL METHODS

Apparatus

Microanalyses of C, H and N were performed by the Technical Services of University of Granada. IR spectra were registered on a Perkin–Elmer 983-G spectrophotometer, from KBr pellets, in the 4000–250 cm^{-1} range. ^1H and ^{13}C NMR spectra were obtained on a Bruker AM-300 apparatus, using DMSO-d_6 as solvent and TMS as internal standard.

Thermoanalytical data were obtained from TG, DTG and DSC curves. These were recorded on a Mettler TA-3000 system provided with a Mettler TG-50 thermobalance and a DSC-20 differential scanning calorimeter. The atmosphere used was a pure air flow (100 ml min^{-1}) for TG studies and static air for DSC runs. The heating rate was 10°C min^{-1} and the sample mass was between 3 and 15 mg.

Preparation of compounds

Both acids utilised in the present work were purchased as the monohydrates from Sigma and used without further purification.

TABLE 1

Analytical data and colour for ammonium, sodium and barium salts of isoorotic and 2-thioisoorotic acids

Compound	C (%)	H (%)	N (%)	Colour
$NH_4(H_2CU) \cdot 1\frac{1}{2}H_2O$	30.50	5.69	20.98	White
	(30.00)	(5.05)	(21.00)	
$NH_4(H_2CTU)$	31.62	4.17	22.84	Yellow
	(31.74)	(3.74)	(22.21)	
$Na(H_2CU) \cdot H_2O$	29.95	2.43	14.01	White
	(30.62)	(2.58)	(14.29)	
$Na(H_2CTU)$	31.02	1.75	14.50	White
	(30.93)	(1.56)	(14.43)	
$Ba(HCU) \cdot 1\frac{1}{2}H_2O$	17.90	1.24	8.38	White
	(18.86)	(1.59)	(8.80)	
$Ba(HCTU) \cdot H_2O$	19.41	0.96	9.21	White
	(18.45)	(1.24)	(8.61)	

Calculated values in parentheses.

The synthesis of ammonium salts as carried out by dissolving the corresponding acid in a concentrated aqueous solution of ammonia. When the resulting solutions were air evaporated, both ammonium salts were isolated. Sodium and barium salts were obtained by adding to an aqueous solution of the corresponding acid (1 mmol), 1 mmol of either $NaHCO_3$ or $Ba(OH)_2 \cdot 8H_2O$ respectively. In the former, the precipitation of compounds takes place after a few days, whereas the barium salts precipitate immediately. All the isolated compounds were filtered off, washed with ethanol and diethyl-ether and air dried. The analytical results and colour are given in Table 1.

RESULTS AND DISCUSSION

Spectral data

Major IR bands of free acids and their ammonium, sodium and barium salts, in hydrated and anhydrous forms, are collected in Table 2. The assignments of bands were carried out by comparison with published data, from both theoretical and practical points of view, for similar pyrimidine derivatives [24–30], their metal complexes [31–36] and rings involving the –NH–CS–NH– group [32,37–39].

All the spectra exhibit a broad band between ca. 3300 cm^{-1} and 2600 cm^{-1} which includes the stretching mode of C–H and N–H bonds. The broadening of this band is smaller in the IR spectra of anhydrous acids and salts since dissociation processes and, consequently, the disappearance of O–H and N–H groups promotes the breaking of hydrogen bonds. Similarly, IR spectra of free acids display a multicomponent and poorly defined band at ca. 2500 cm^{-1} assigned to ν(O–H) of a carboxylic acid in its dimer form. This band does not appear in the IR spectra of salts.

The range between 1750 cm^{-1} and 1500 cm^{-1} includes, in all cases, many bands assigned to ν(C=O), δ(O–H), δ(N–H) and ν(C=C) modes. These bands are also progressively better resolved when O–H and N–H bonds disappear. Similarly, the stretching ν(C=O) band of the carboxylic group may be overlapping with one corresponding to uracil carbonyl groups, whereas ν(C–O) appears between 1200 cm^{-1} and 1180 cm^{-1}. The transformation of a carboxylic group into a carboxylate anion results the shift of these two bands to lower and upper wavenumber respectively (Table 2). From bands corresponding to the C=S bond, only those assigned to thioamide I, ν(N–C=S) + ν(C=C) + δ(N–H), and thioamide III, ν(N–C=S) + ν(C=S), were identified.

Finally, according to reported data [40], both acids in the solid phase are preferably in their diketonic form; it is not possible to follow from IR data the enolisation processes that must take place in the formation of salts.

TABLE 2

Tentative assignments of major IR bands (cm^{-1})

Compound	ν(O–H)	ν(N–H) ν(C–H)	ν(C=O)	δ(O–H)	ν(C=C)	ν(NCS) [a]	ν(NCS)+ν(CS) [b]	ν(C–O)
$H_3CU \cdot H_2O$	3469[c], 2400[d]	3300–2600[e]		1710–1600[e]		–	–	1186
H_3CU	2590[d]	3300–2700[e]	1692[d]	–[d]	1616	–	–	1199
$NH_4(H_2CU) \cdot 1\frac{1}{2}H_2O$	3497[c]	3400–2500[e]	1647[f]		1622	–	–	1383[g]
$NH_4(H_2CU)$	–	3400–2400[e]	1694[f]	–	1622	–	–	1383[g]
$Na(H_2CU) \cdot H_2O$	3508[c]	3300–2600[e]	1738	1688[f]	1607	–	–	1381[g]
$Ba(HCU) \cdot 1\frac{1}{2}H_2O$	3413[c]	3300–2600[d]	1622[f]	1641[f]		–	–	1402[g]
$Ba(HCU)$	–	3300–2700[e]		–	1622[f]	–	–	1386[g]
$H_3CTU \cdot H_2O$	3498[c], 2531[d]	3300–2700[e]	1676[f]		1616	1561	1176[h]	–[i]
H_3CTU	2533[d]	3300–2700[e]	1730, 1706	1677[d]	1603	1570	1180, 1161	1202
$NH_4(H_2CTU)$	–	3300–2600[e]	1699	–	1614	1536	1191, 1146	1437[g]
$Na(H_2CTU)$	–	3300–2700[e]	1701[f]	–	1582	1558	1202, 1174	1389[g]
$Ba(HCTU) \cdot H_2O$	3505[c]	3300–2700[e]		1660[f]		1545	1199, 1173	1385[g]
$Ba(HCTU)$	–	3300–2700[e]	1639[f]	–	1580	1542	1201, 1170	1370[g]

[a] Thioamide I; [b] thioamide III; [c] water; [d] carboxylic acid; [e] few bands; [f] broad band; [g] symmetric stretching of carboxylate group; [h] ν(C–O) of carboxylic acid is included; [i] included in the band corresponding to thioamide III.

TABLE 3

NMR chemical shifts δ, (ppm)

Compound [a]	C2	C4	C5	C6 [b]	C5′	H6	N1–H	N3–H
H₃CU	150.60	163.40	101.14	150.33	165.18	8.26	12.10 [c]	
NH₄(H₂CU) [d]	158.31	166.46	96.88	161.30	168.33	8.32	– [e]	– [e]
H₃CTU	160.54	163.16	106.05	147.75	176.27	8.01	12.96 [c]	
NH₄(H₂CTU) [d]	166.08	166.43	102.13	156.17	184.05	8.16	– [e]	– [e]
Na(H₂CTU)	162.81	166.62	106.12	158.95	168.64	8.30	– [e]	– [e]

[a] Sodium isoorotate and both barium salts are not soluble in DMSO.
[b] Positive signal in DEPT spectrum.
[c] Broad.
[d] Signal corresponding to ammonium hydrogen atoms appears at 5.75 ppm.
[e] not observed.

Spectral characterisation has been completed with the study of ^{1}H and ^{13}C NMR spectra. Values of the chemical shifts are given in Table 3. The assignation of signals was carried out according to the literature data [41] and DEPT spectra. In the majority of cases, the ^{13}C NMR chemical shifts are in the order C5′ > C4 > C2 > C6 > C5.

Thermal study

The thermal behaviour of isolated compounds has been investigated from their TG and DSC plots, which are shown in Figs. 1 and 2.

From these figures, it can be observed that the thermal degradation of these compounds take place through two major processes: dehydration and pyrolytic decomposition.

Dehydration processes

Thermoanalytical data concerning dehydration processes are given in Table 4.

The occurrence of these processes has been made manifest by IR spectra of samples heated until the weight loss effect ends. These spectra reveal the disappearance of bands assigned to water vibration modes (ν(O–H) and δ(O–H)) in the spectra of hydrated species (Table 3). Figures 1 and 2 also show that, with the exception of ammonium and sodium isoorotates (Figs. 1c and 2a), the water loss takes place in only one step, the enthalpy values for these processes being in good accordance with those expected for the breaking of hydrogen bonds.

Pyrolytic processes

Once dehydrated, free acids show in their TG plots (Figs. 1a and 1b) two sharp weight loss effects centred at about 300 and 370 °C and a third at
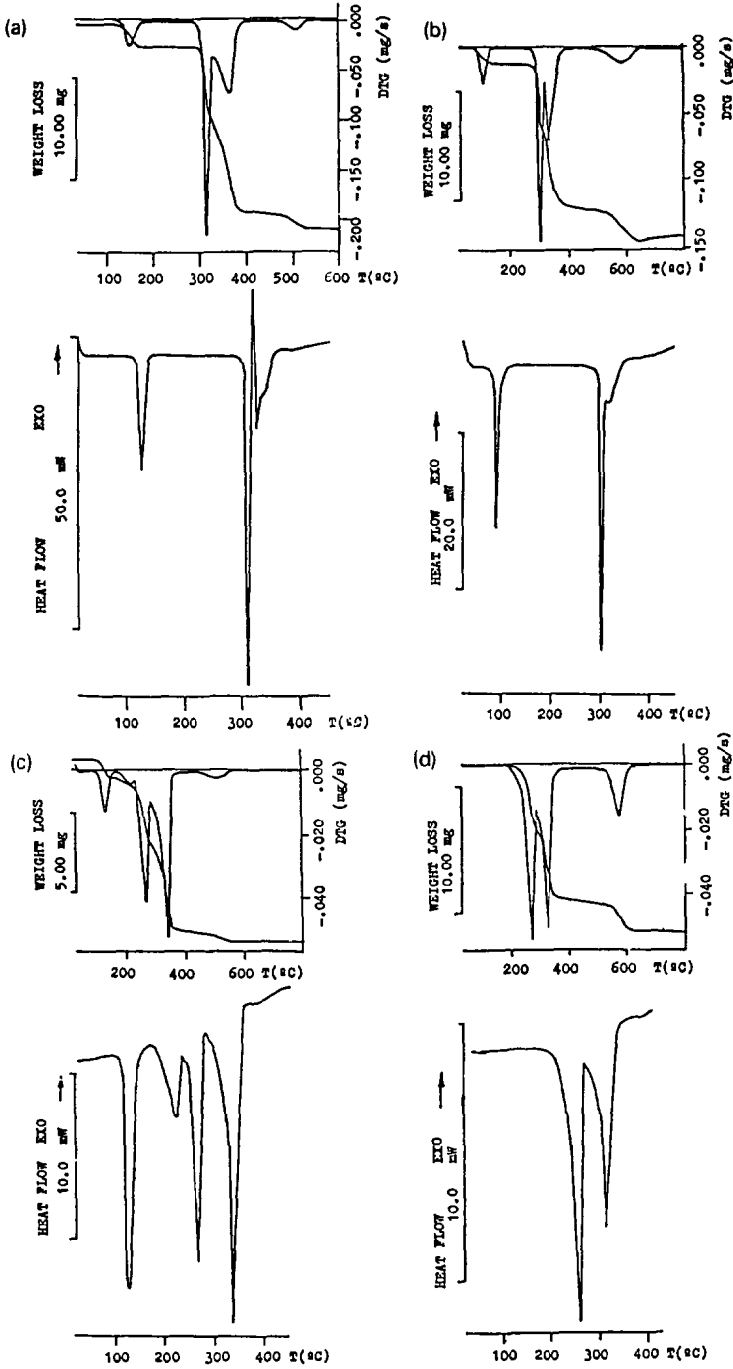
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Fig. 1. TG and DSC plots for (a) $H_3CU \cdot H_2O$, (b) $H_3CTU \cdot H_2O$, (c) $NH_4(H_2CU) \cdot 1\frac{1}{2}H_2O$ and (d) $NH_4(H_2CTU)$.
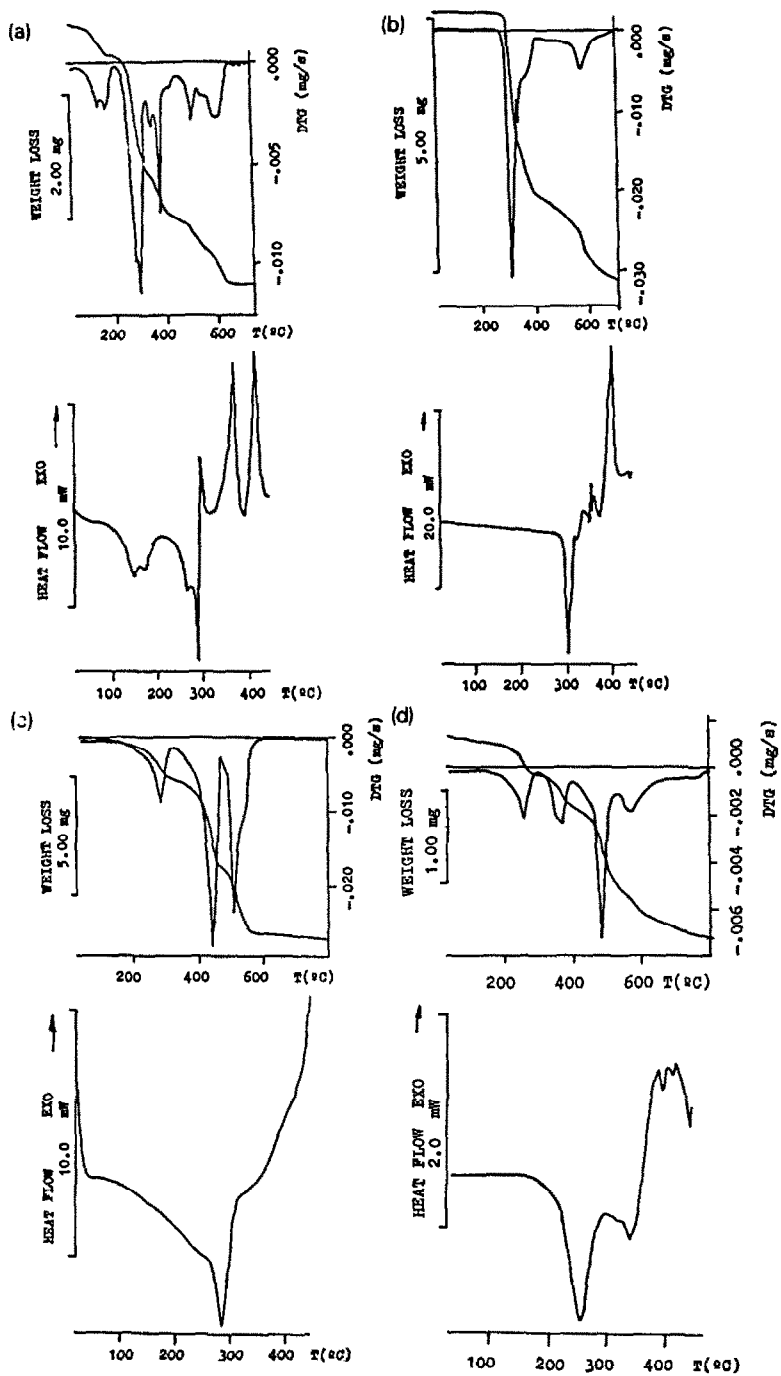
Fig. 2. TG and DSC plots for (a) Na(H$_2$CU)·H$_2$O, (b) Na(H$_2$CTU), (c) Ba(HCU)·1$\frac{1}{2}$H$_2$O and (d) Ba(HCTU)·H$_2$O.

TABLE 4

TG and DSC data for dehydration processes

Compound	Weight loss (%)	ΔT (TG) (°C)	T_{peak} (DSC) (°C)	ΔH kJ (mol $H_2O)^{-1}$
$H_3CU \cdot H_2O$	9.9 (10.34)	120–180	127	52
$H_3CTU \cdot H_2O$	8.9 (9.40)	80–150	86	51
$NH_4(H_2CU) \cdot 1\frac{1}{2}H_2O$	9.8 (9.00)	100–160	128	55
$NH_4(H_2CU) \cdot \frac{1}{2}H_2O$	5.3 (4.50)	175–250	222	41
$Na(H_2CU) \cdot H_2O$	9.3 (9.18)	70–230	148, 175	44
$Ba(HCU) \cdot 1\frac{1}{2}H_2O$	7.8 (8.48)	150–325	288	27
$Ba(HCTU) \cdot H_2O$	6.1 (5.53)	180–300	257	53

Calculated values in parentheses.

500 °C (H_3CU) and 600 °C (H_3CTU). The first two effects are reflected in DSC diagrams as two overlapping endothermic effects; the first of these could be attributed to the melting of anhydrous species. However, in this temperature range, 5-carboxypyrimidines undergo decarboxylation processes that are endothermic also. Moreover, the weight loss that takes place in TG plots is greater than that expected for the loss of carbon dioxide. This permits us to affirm that, in the first effect, melting, decarboxylation and partial pyrolysis take place consecutively, it not being possible to calculate the associated enthalpy values for each process. Decarboxylation processes have been identified from IR spectra of intermediate species, since the bands assigned to ν(C–O) of the carboxylic group (ca. 1200 cm^{-1}) do not appear. On the other hand, melting processes have been followed in an open capillary, the melting points being 280 °C (H_3CU) and 283 °C (H_3CTU). Just after the melting process, a darkening of samples takes place, which is indicative that partial pyrolysis occurs.

Also, the similarity of TG and DSC plots of free acids as well as IR spectra of both heated samples at 415 °C (only a broad band centred about 1600 cm^{-1}) indicates that the thermal degradation of both compounds takes place in the same way. This conclusion is in accordance with the results obtained from mass spectrometry diagrams [42]. Pyrolytic processes end at 520 °C (H_3CU) and 625 °C (H_3CTU), no residue remaining in the crucible.

In the same manner, TG and DSC plots of ammonium salts (Figs. 1c and 1d) show that the thermal decomposition and both anhydrous compounds is very similar. The assignation of endothermic effects has been attempted by means of IR spectral methods. From the data obtained, it may be suggested that in the first effect, as in the case of free ligands, decarboxylation and partial pyrolysis take place. Also, the loss of CO_2 can easily be observed since the IR spectra of samples heated at about 300 °C do not exhibit the band assigned to ν_s(COO$^-$) of carboxylate group (ca. 1380 cm^{-1}). An analogous comment would hold for the thermal behaviour of sodium salts

(see Figs. 2a and 2b). In these cases, the combustion of organic matter appears to be reflected in DSC plots as several exothermic effects.

At the end of pyrolysis, ammonium salts leave no residue, whereas sodium salts leave the following residues, whose nature has been verified by IR spectroscopy:

$$Na(H_2CU) \cdot H_2O \rightarrow Na_2CO_3 + Na_2O$$
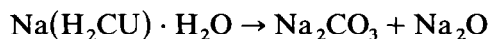
(weight loss found, 79.9%; weight loss calculated, 72.96% for Na_2CO_3, 84.19% for Na_2O);

$$Na(H_2CTU) \rightarrow Na_2SO_4$$
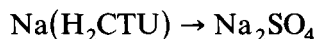
(weight loss found, 65.1%; weight loss calculated, 63.40% for Na_2SO_4).

Once dehydrated, thermal decomposition of barium salts does not seem to start with decarboxylation, since in view of their TG and DSC plots (Figs. 2c and 2d), the only endothermic effects that appear have been attributed, as verified by means of IR spectroscopy, to water loss, being exothermic effects due to the combustion of the organic moiety. The absence of carbon dioxide loss is supported by the observance of a pyrolytic residue, since in both cases the major component of this residue is barium carbonate. Found and calculated weight losses as well as the nature of the thermal degradation products are the following:

$$Ba(HCU) \cdot 1\tfrac{1}{2}H_2O \rightarrow BaCO_3$$
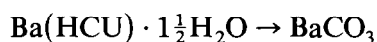
(weight loss found, 37.1%; weight loss calculated, 38.02% for $BaCO_3$);

$$Ba(HCTU) \cdot H_2O \rightarrow BaCO_3 + BaSO_4$$
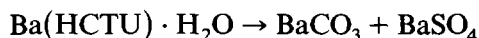
(weight loss found, 36.2%; weight loss calculated, 39.35% for $BaCO_3$, 26.29% for $BaSO_4$).

As is commonly found for other 2-thioisoorotate metal complexes [42], the residue of barium salt contains a small amount of the corresponding sulphate.

REFERENCES

1 I. Lieberman, A. Kornberg and E.S. Simms, J. Biol. Chem., 215 (1955) 403.
2 D.D. Genchev, Dokl. Bolg. Akad. Nauk, 23 (1970) 435.
3 Malaya Meditsinskaya Entsiklopediya (Small Medical Encyclopedia), Vol. 6, Izd. Sovetskaya Entsiklopediya, Moscow, 1967, p. 970.
4 N.K. Davidenko and N.N. Zinich, Russ. J. Inorg. Chem., 24 (1979) 891.
5 G.S. Pandey, P.C. Nigam and U. Agarwala, Indian J. Chem. A, 15 (1977) 537.
6 A.K. Singh and R.P. Singh, Indian J. Chem. A, 17 (1979) 469.
7 G.S. Pandey, Indian J. Chem. A, 27 (1988) 5.
8 D. Lalart, J. Guillerez, G. Dodin and J.E. Dubois, J. Chem. Soc., Perkin Trans. 2, (1981) 1057.
9 P. Arrizabalaga, P. Castan and J.P. Laurent, J. Inorg. Biochem., 20 (1984) 215.

10 P. Arrizabalaga, P. Castan and J.P. Laurent, Inorg. Chim. Acta, 92 (1984) 203.

11 T. Solin, K. Matsumoto and K. Fuwa, Bull. Chem. Soc. Jpn., 54 (1981) 3731.

12 M. Sabat, D. Zglinska and B. Jezowska-Trzebiatowska, Acta Crystallogr. B, 36 (1980) 1187.

13 I. Mutikainen and P. Lumme, Acta Crystallogr. B, 36 (1980) 2233.

14 P. Arrizabalaga, P. Castan and F. Dahan, Inorg. Chem., 22 (1983) 2245.

15 I. Mutikainen; Finn. Chem. Lett., 193 (1985).

16 A. Karipides and B. Thomas, Acta Crystallogr. C, 42 (1986) 1705.

17 I. Mutikainen, Inorg. Chim. Acta, 136 (1987) 155.

18 M. Moreno-Carretero and J.M. Salas-Peregrin, J. Therm. Anal., 30 (1985) 377.

19 J. Ruiz-Sánchez, E. Colacio-Rodriguez, J.M. Salas-Peregrín and M.A. Romero-Molina, J. Anal. Appl. Pyrolysis, 9 (1986) 159.

20 J.M. Salas-Peregrín, M.N. Moreno-Carretero and E. Colacio-Rodriguez, Can. J. Chem., 63 (1985) 3573.

21 J.M. Salas-Peregrín, M.N. Moreno-Carretero, J.D. López-González and M.A. Romero-Molina, Spectrochim. Acta A, 42 (1986) 607.

22 G. Cruz-Bermúdez, A. García-Rodríguez, M. Moreno-Carretero, J.M. Salas-Peregrín and C. Valenzuela-Calahorro Monatsh. Chem., 118 (1987) 329.

23 F. Hueso-Ureña, M.N. Moreno-Carretero, J.M. Salas-Peregrín, C. Valenzuela-Calahorro and G. Alvarez de Cienfuegos, Thermochim. Acta, 133 (1988) 341.

24 W.D. Bowman and T.G. Spiro, J. Chem. Phys., 73 (1980) 5482.

25 T.P. Lewis, H.T. Miles and E.D. Becker, J. Phys. Chem. 88 (1984) 3253.

26 E. Taillandier, J. Liquier and J.A. Taboury, in R.J.H. Clark and R.E. Hester (Eds.), Advances in Infrared and Raman Spectroscopy, Vol. 12, Wiley, New York, 1985, Chap. 2.

27 S. Aruna and G. Shanmugam, Spectrochim. Acta A, 41 (1985) 531.

28 M. Ghomi, R. Letellier, E. Taillandier, L. Chinsky, A. Laigle and P.Y. Turpin, J. Raman Spectrosc., 17 (1986) 249.

29 L. Harsanyi, P. Csaszar, A. Csaszar and J.E. Boggs, Int. J. Quantum Chem., 29 (1986) 799.

30 P. Csaszar, L. Harsanyi and J.E. Boggs, Int. J. Quantum Chem., 33 (1988) 1.

31 G.S. Pandey, P.C. Nigam and U. Agarwala, J. Inorg. Nucl. Chem., 39 (1977) 1877.

32 E.S. Raper, Coord. Chem. Rev., 61 (1985) 115.

33 R. Battistuzzi and G. Peyronel, Spectrochim. Acta A, 36 (1980) 113.

34 J.F. Villa and H.C. Nelson, J. Indian Chem. Soc., 55 (1978) 631.

35 M. Goodgame and K.W. Johns, Inorg. Chim. Acta, 46 (1980) 181.

36 D. Kovala-Demertzi, J.M. Tsangaris, H.O. Desseyn and B.J. Van der Veken, Bull. Soc. Chim. Belg., 96 (1987) 1.

37 P. Castan, Trans. Met. Chem., 6 (1981) 14.

38 P. Castan and J.P. Laurent, Trans. Met. Chem., 5 (1980) 154.

39 B. Singh and P. Takur, J. Inorg. Nucl. Chem., 36 (1974) 1735.

40 M. Goodgame and D.A. Jakubovic, Coord. Chem. Rev., 79 (1987) 97.

41 P.D. Ellis, R.B. Bruce-Dunlap, A.L. Pollard, K. Seidman and A.D. Cardin, J. Am. Chem. Soc., 95 (1973) 4398.

42 F. Hueso-Ureña, Ph.D. thesis, University of Granada, 1990.