Pak. j. sci. ind. res., vol. 36, nos. 2-3, Feb.-Mar. 1993

DISSOCIATION CONSTANTS OF ARYLAZO OROTIC ACID COMPOUNDS AND STABILITY CONSTANTS OF THEIR COMPLEXES

EKRAM A. KHALIL, MAMDOUH S. MASOUD AND ADEL M. EL-MERGHANY

Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

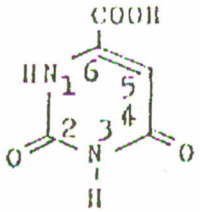
(Received July 7, 1992; revised January 30, 1993)

Synthesis of new arylazo orotic acids with different functional groups has been carried out. pK_L and log K_c values were evaluated. Solvent effects on the thermodynamic parameters of dissociation were discussed. The data were explained from the electronic character of the substituents.

Key words: Synthesis, Arylazo orotic acid, Complexes.

Introduction

The N-heterocyclic compounds containing amide linkages are widely used in medicine, principally as hypnotic drugs [1].



(1)

Orotic acid, a key compound (I) involved in the de novo biosynthesis of pyrimidine bases of nucleic acids in living organisms [2-4], is like a cyclic amide. The overall process of enzymatic phosphoribosyl pyrophosphate ultimately requires an unsubstituted N (1) nitrogen atoms [5]. Metals ions make orotic acid available in the form of the reactive N (3) H dianion where N (1) is unsubstituted, thus containing to the phosphoribosylation at the N (1) site [6]. It is also known to display bacteriostatic and cytostatic properties. Oral cosmetic contains an active ingredient of a mixture at least of magnesium and potassium orotates for the treatment of skin, hair and nails. The cosmetics prevent pigmentation, wrinkles and protect against the effect of the sun [6].

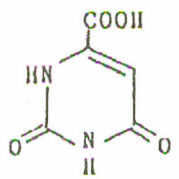
The coordinating behaviour of some new arylazo orotic acid compounds (B) is described, based on the following items:

(i) Evaluation of the dissociation constants of the free azo compounds by spectrophotometric and potentiometric methods at different temperatures. The effect of ethanol and dioxane on the pK values is discussed. The thermodynamic parameters of dissociation are evaluated. The data are explained in the light of molecular structure of the compounds.

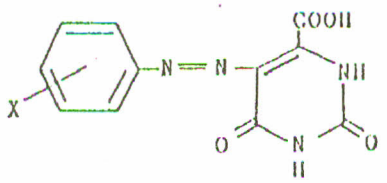
(ii) The stability constants of cobalt, nickel and copper complexes are also aimed to be evaluated.

Experimental

Orotic acid (A) was supplied from BDH. Dyestuff-chromogenic azo orotic acid compounds (B) were prepared by the usual diazotization process [7]. The corresponding amines (0.1 mole) were dissolved in 0.2 mole HCl and 25 ml distilled water. The hydrochloride compounds were diazotized below 5° with 0.1 mole $NaNO_2$ solution and 20 ml distilled water. The diazonium chlorides were coupled with an alkaline solution of (0.1 mole NaOH) orotic acid. The crude dyes were filtered and were crystallized from ethanol.



(A)



(B)

X = II, *p*-CH_3, *o*-OH, *p*-Br, *o*-COOH, *m*-COOH, *p*-COOH.

Acidity and stability constants were determined by using acid-base titration technique. A 10^{-2} M stock solution of the organic compound was prepared. A standard 10^{-2} M KOH solution was prepared in CO_2 free distilled water. 0.5 M KCl was used as a supporting electrolyte. 0.1 M stock metal salt solutions were prepared and the exact concentration was determined by direct complexometric titration with EDTA [8]. A stream of purified nitrogen gas was passed through the solution during the whole titration. BDH spectroscopic-quality solvents (dioxane and ethanol) were used. The correction factor, δ, for measuring the pH values in different dioxane-water and ethanol-water concentration was calculated [9].

The dissociation constants of the organic compounds were determined by introducing the appropriate volume of the organic compound into the titration cell in presence of 5 ml 0.5 M KCl solution and 75% (v/v) ethanol-water at different temperatures 27-45° and 75% (v/v) dioxane-water at 27°. The

same potentiometric titration experiment was applied for studying the complex equilibria in 75% (v/v) ethanol-water. The reaction mixture was titrated against 2.58 x 10^{-3} M KOH. The Cole-Palmer pH-meter Model 60648 was used. The electrode system was calibrated before and after each titration using standard buffers of pH's 4.01 and 9.14. The titrations were carried out in 150 ml thermostated cell. The cell compartment was kept constant at desired temperature by using a thermostate Model U10. The electronic absorption spectra were measured using Perkin Elmer spectrophotometer Model Lambda 4B covering a range from 190-900 nm.

Results and Discussion

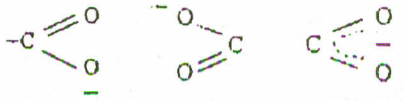
Dissociation constants of the ligands. Three basic spectrophotometric methods are applied for this purpose: half height [10], Collcter [11] and modified limiting absorption [12]. The potentiometric measurements depend on the evaluation of the average number of proton associated with the ligand [13], $\bar{n}_A$. The plotting of $\bar{n}_A$ values against pH gave three pK's values (for orotic acid and its 5-(substituted phenylazo) orotic acid compounds except the *p*-Br derivative. The latter gives two pK's values. The data are collected in Table 1. The Point-Wise [14] and basic method of calculation constructed by Martell [15] were used for the same purpose, where concordant results are obtained. The plot of the log $\bar{n}_A$ ratio versus pH gives the required pK values (Table 1).

TABLE 1. pK VALUES FOR THE OROTIC ACID AND 5-(SUBSTITUTED ARYLAZO)OROTIC ACID IN DIFFERENT SOLVENTS.

Compound	Pk in dioxane-water 75%			Pk in ethanol-water 75%	
	$\bar{n}_A$-pH	Point-wise method	Algebric	$\bar{n}_A$-pH	Point-wise method
Orotic acid	4.76	4.86	4.79	5.55	5.52
	9.56	9.66	9.61	10.30	10.30
	11.66	11.66	11.67	11.20	11.20
5-(arylazo) orotic acid	3.96	3.92	3.86	4.50	4.53
	4.91	4.91	4.91	10.20	10.22
	10.36	10.36	10.31	12.10	12.10
5-(*p*-methyl-arylazo)orotic acid	5.41	5.40	5.48	7.60	7.60
	9.72	9.70	9.66	10.20	10.15
	11.41	11.40	11.41	11.10	11.10
5-(*p*-bromo-aryl-azo)orotic acid	4.26	4.31	4.26	10.10	10.14
	9.41	9.46	9.44	11.45	11.45
	11.91	11.93	11.92	---	---
5-(*o*-hydroxy-arylazo)orotic acid	4.71	4.69	4.65	5.40	5.47
	9.90	9.90	9.93	10.50	10.50
	12.06	12.06	12.08	11.78	11.80
5-(*o*-carboxy-arylazo)orotic acid	8.20	8.15	8.09	8.35	8.34
	11.55	11.55	11.56	10.65	10.67
	12.60	12.60	12.65	11.40	11.40
5-(*m*-carboxy-arylazo)orotic acid	4.36	4.35	4.32	4.15	4.16
	9.26	9.30	9.30	9.35	9.43
	11.55	11.60	11.55	11.35	11.32
5-(*p*-carboxy arylazo)orotic acid	9.25	9.20	9.21	9.10	9.10
	11.70	11.70	11.74	10.65	10.67
	12.60	12.60	12.60	11.30	11.32

The spectral bands are pH dependent and the shape of the band envelope can be correlated to the nature of the absorbing species depending on the electronic character of the substituent, to some extent. Earlier literature [16-18] concerned with orotates where the state of ionization of these molecules at physiological pH controlled the importance of these compounds as drugs. The 208-214 nm region of the uv spectra is mainly due to π- π^*. electronic transition and that of 277-288 nm spectra is considered as n-π^*. In solution of sufficient (H^+), the proton is bound symmetrically to both N atoms [19], i.e. acts as a single basic site [20] or in the form of π-complex [21] and the azo group can act as a proton acceptor. The aromatic azo compounds are resonance stabilized. The hydrazone-azo tautomerism can be strongly influenced by synergetic tautomerism in another portion of the molecule [22]. In the pH range 3-9, orotic acid exists in aqueous solutions mainly as the orotate anion (I). The N(3)H with N(1) unsubstituted is obtained by abstraction of a second proton (pK =9.45) exists together with N (1)H tautomer [23]. So the mechanism of lactam-lactim tautomerism is represented as follows:

The pK_2 of orotic acid refer to proton dissociation from a hydroxyl group, or from a -NH-C(=O)- structure. The following structures illustrate how stabillization by hydrogen-bonding would favour keto $\rightleftharpoons$ enol formation for orotic acid in the region of pK_2 [24]. The azo group in the reactive position 5 of orotic acid affects the mode of ionization to some extent. In general all the azo compounds under investigation undergo a regular bathochromic shift on increasing the pH as a result of proton elimination. From half height method (Table 2), all the compounds except those containing *p*-COOH, m-COOH groups are characterized by pK value lies in the range 9.30-10.1 due to ionization of N(3)H group. The latter two compounds gave pK_2 value equals to 3.5 and 4.4 respectively. Such value may due to ionization of the carboxy group of the azo phenyl ring and not for carboxy of orotic acid nuclei. Such value is not detected in *o*-carboxy compounds, where the -COOH group is strongly bonded to the azo group through intramolecular hydrogen bond [25]. The extra pK value for the *p*-COOH compound (7.0) is assigned to its presence in different resonating structures. For this compound the carboxy group is more donor rather than acceptor [26]. Such abnormal behaviour is detected in some schiff base compounds containing carboxy group.



As a general trend, the compounds with electron attracting group, e.g. -*p*-Br, are with low pK value compared to the unsubstituted compound (arylazo). The electron donor groups (e.g. *p*-CH_3) lead to increase the pK values. In general, no regular trend could be recorded on relating the pK values with the Hammett constants, where scattering correlation exists. The slope of the modified limiting absorption method merits some comments. Three groups of compounds are classified from this view:

(1) A group with a slope of 1 obtained for orotic acid and phenylazo orotic acid compounds, denotes that the mechanism of dissociation gives only one H^+, detected by only pK_1 value N(3)H.

$$H_3L \rightleftharpoons H^+ + H_2L^-$$

(2) A group with a slope =2 is obtained from *p*-tolylazo orotic acid, *p*-bromoaryl azo orotic acid *o*-carboxy aryl azo orotic acid and *p*-carboxy aryl azo orotic acid. So the mechanism of dissociation could be formulated as:

$H_3L \rightleftharpoons 2H^+ + HL^-$ (for *p*-bromo and *p*-tolyl azo compounds)

$H_4L \rightleftharpoons 2H^+ + H_2L^{-2}$ (for *o*-carboxyl aryl azo compound)

$H_4L \rightleftharpoons H^+ + H_3L^-$

$H_3L \rightleftharpoons H^+ + H_2L^{-2}$ (for *o*-carboxyl aryl azocompound)

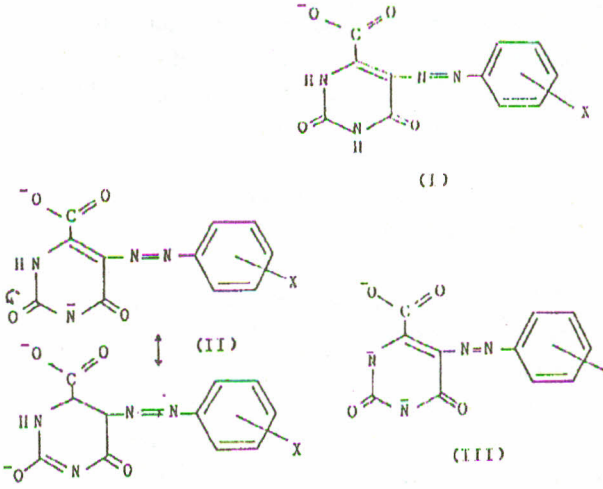
(3) A group with a slope =3 is obtained from both o-hydroxy and *m*-carboxy aryl azo orotic acids.

$H_4L \rightleftharpoons 3H^+ + HL^{-3}$ (for the *o*-hydroxy)

$H_4L \rightleftharpoons H^+ + H_3\bar{L}$ (for *o*-carboxyl aryl azo compound)

$H_3\bar{L} \rightleftharpoons 2H^+ + HL^{-3}$

Orotic acid and all the substituted phenylazo orotic acids in 75% dioxane-H2O and 75% ethanol-H2O gave three pK's values based on potentiometric measurements. The first ionization is due to the carboxylic group of orotic nuclie (I). Based on the second ionization, the ligand exists as the orotate anion (I). N(3) (H) [22] (II) in which N(1) nitrogen is unsubstituted is obtained by abstraction of a second proton.



The pK values obtained potentiometrically in presence of 75% v/v ethanol-H_2O and 75% v/v dioxane-H_2O media, where the compounds with *p*- and *o*-carboxy groups have higher pK's values, except the pK_2 and pK_3 in ethanol due to the COO-group in the *o*- and *p*- positions can act as electron donor rather than electron acceptor. Abnormal behaviour is obtained for the compounds containing *o*-OH and *p*-Br groups in 75% v/v ethanol -H_2O where high pK_2 values are obtained than those with -CH_3 group. The electronegativity property of the substituent is a major factor for controlling the behaviour of these compounds.

Complex formation studies. The acid base properties of the free ligand facilitate the investigation of the coordinating behaviour of these ligands towards cobalt (II), nickel (II) and copper (II). Comparing the pH titration curves of the free ligands with that of the complex solutions reveals a drop in pH,

TABLE 2. SUMMARY FOR THE PK VALUES OF 5-(SUBSTITUTED ARYLAZO) OROTIC ACID BY SPECTRAL METHOD.

Compound	λ_{max} (nm)	Isobestic point (nm)	Half height			Modified limiting absorption			Colleter	
			pK_1	pK_2		pK_1	pK_2	slope	pK_1	pK_2
Orotic	210, 280	286	9.30			9.25		1	9.39	
R = H	214, 283	252, 295	9.50			9.40		1	9.32	
p = CH_3	210, 279, 347	253	9.55			9.50		2	9.60	
p-Br	209, 278	—	9.45			9.50		2	9.70	
o-OH	212, 279	295	9.30			9.20		3	9.40	
o-COOH	208, 277	293	9.65			9.70		2	9.84	
m-COOH	288	297	4.40	9.60		4.50	8.65	3	4.35	9.45
p-COOH	285	—	3.50	7.00	10.10	6.30	9.70	2	5.10 (7.10)	10.03

TABLE 3. THERMODYNAMIC PARAMETERS OF IONIZATION OF THE OROTIC ACID AND SUBSTITUTED ARYLAZO OROTIC ACID.

Compound	pK 27°	30°	35°	40°	45°	ΔG_{27}°C K. cal/mol.	ΔH K. cal/mol.	Δ S e.u.
Orotic acid	5.55	5.80	5.90	6.05	6.15	7.66	-9.20	-56.19
	(10.35)	(10.20)	(10.10)	(9.85)	(9.75)	(14.28)	(14.92)	(2.12)
	(11.20)	(11.08)	(10.95)	(10.80)	(10.68)	(15.45)	(11.34)	(-13.71)
5-(arylazo) orotic	4.55	4.55	4.55	4.55	4.55	—	—	—
acid	(10.20)	(9.95)	(9.45)	(9.22)	(9.10)	(14.07)	(31.98)	(59.68)
	(12.10)	(11.78)	(11.20)	(10.75)	(10.40)	(16.69)	(45.12)	(94.75)
5-(*p*-methylaryl-	7.60	7.47	7.40	7.30	7.20	10.49	8.76	-5.75
azo)- orotic acid	(10.20)	(10.00)	(9.90)	(9.82)	(9.75)	(14.07)	(7.42)	(-22.18)
	(11.10)	(11.00)	(-10.90)	(10.80)	(10.75)	(15.31)	(9.20)	(-20.40)
5-(*p*-bromoarylazo)	10.10	9.95	9.70	9.25	9.10	13.94	24.86	36.42
-orotic acid	((11.45)	(11.30)	(11.20)	(10.90)	(10.75)	(15.60)	(16.32)	(1.73)
5-(*o*-hydroxyaryl-	5.45	5.23	5.10	4.85	4.70	7.52	20.12	42.01
azo)- orotic acid	(10.55)	(10.40)	(9.95)	(9.80)	(9.57)	(14.56)	(24.76)	(34.02)
	(11.77)	(11.50)	(11.23)	(10.87)	(10.55)	(16.24)	(28.68)	(41.46)
5-(*o*-carboxyaryl-	8.35	8.35	8.35	8.35	8.35	—	—	—
azo) orotic acid	(10.65)	(10.55)	(10.42)	(10.20)	(10.10)	(14.69)	(13.22)	-(-4.91)
	(11.40)	(11.25)	(11.10)	(10.87)	(10.75)	(15.73)	(13.80)	(-6.44)
5-(*m*-carboxyaryl-	4.15	4.27	4.35	4.44	4.60	5.72	-11.50	-57.42
azo)-orotic acid	(9.49)	(9.40)	(9.30)	(9.16)	(9.07)	(13.09)	(10.07)	(-10.07)
	(11.35)	(11.07)	(10.90)	(10.70)	(10.45)	(15.66)	(21.25)	(17.03)
5-(*p*-carboxyaryl-	9.10	9.10	9.10	9.10	9.10	—	—	—
azo)-orotic acid	(10.65)	(10.57)	(10.48)	(10.37)	(10.30)	(14.69)	(8.28)	(-21.39)
	(11.33)	(11.22)	(11.10)	(10.90)	(10.73)	(15.63)	(14.65)	(-3.28)

( ) = pk_2 and pk_3 respectively.

TABLE 4. STOICHIOMETRY AND FORMATION CONSTANTS OF THE COMPLEXES AT 27°C .

Ligand	Co(II) Stoichio-metry	log K $\bar{n}$ pL	Point-wise method	Ni(II) Stoichio-metry	log K $\bar{n}$ pL	Point-wise method	Cu(II) Stoichio-metry	log K $\bar{n}$ pL	Point-wise method
Orotic acid	1:1	9.03	8.90	1:1	6.90	6.45	1:1	10.80	10.84
	1:2	5.80	5.84	1:1	—	—	1:1	8.20	8.10
	1:3	—	—	1:3	—	–	1:3	4.65	4.65
5-(arylazo)orotic	1:1	8.80	8.70	1:1	9.50	9.40	1:1	9.00	9.05
5-(*p*-methylaryl-	1:1	13.35	13.20	1:1	16.00	16.00	1:1	14.55	14.50
azo)orotic acid	1:2	—	–	1:2	—	—	1:2	10.95	10.85
5(*p*-bromoarylazo)-	1:1	9.50	9.45	1:1	7.80	7.85	1:1	10.80	—
orotic acid	1:2	6.80	6.80	1:2	—	—	1:2	9.70	9.85
5-(*o*-hydroxyaryl-	1:1	9.50	9.55	1:1	8.50	8.55	1:1	10.75	10.79
azo) orotic acid	1:2	6.95	7.00	1:2	—	—	1:2	9.15	9.17
							1:3	5.50	5.70
5-(*o*-carboxyaryl-	1:1	13.50	13.50	1:1	15.25	15.25	1:1	14.45	14.50
azo) orotic acid							1:2	10.75	10.65
5-(*m*-carboxyaryl-	1:1	10.65	10.65	1:1	10.40	10.50	1:1	12.10	12.15
azo) orotic acid	1:2	8.35	8.30				1:2	10.70	10.65
							1:3	8.65	8.65
5-(*p*-carboxyaryl-	1:1	12.45	12.35	1:1	11.40	11.40	1:1	14.20	14.25
azo) orotic acid							1:2	10.05	10.05

indicating that the mechanism of complexation is based on hydrogen ion liberation. It is apparent that the ligands under investigation, are of stronger coordinating ability. The pK values of the free ligand are strongly affected on complexation. The pH measurements during titration of the solution of chelating agent in presence and in absence of metal ions with alkali could be used to calculate the free ligand exponent PL, the degree of formation of the system, n, and hence the stability constants of the metal ligand complexes present. Plotting the $\bar{n}$ values versus PL, the log K_1, log K_2 and log K_3 values are recorded at the PL values equivalent to $\bar{n}$ = 0.5, 1.5 and 2.5 respectively. However concordant results were obtained on applying the point wise calculation methods [14]. On plotting log $\bar{n}$ function versus PL, straight lines are obtained from which log K value are computed. The data are collected in Table 3.

An attempt was made to relate the acid dissociation constants of the ligands and the stability constants of their complexes [27]. The linearity between log K_1 and pK_1 could be checked from the relation:

$$\log K_1 = a\, pK_1 + b$$

where a and b are constants. The slope "a" of log K -pK plot would be unity if the bonding was similar in both ligand and the complex, and deviation from unity results from metal complexes with π-bonding. The latter is apparent for cations function as π- electron donors, and a will exceed unity for cations act as π-electron acceptor. The slope for 1:1 cobalt complexes derived from phenylazo orotic acid and its *o*-hydroxy and *p*-tolyl azo compounds 1.4. This points to that cobalt acts as π-electron acceptor. Similar slope is obtained for 1:1 copper complexes derived from orotic acid, phenylazo orotic acid and its *o*-hydroxy and *o*-carboxy derivatives. Also 1:2 copper complex series are present from orotic acid, and its *o*-hydroxy, *o*-carboxy and *p*- bromo compounds. Such plots for the 1:2 copper complexes is 9.4. This suggests that in spite that copper acts as π-electron acceptor, but the ligands are strongly attached to the metals and the charge density on the metal is high. On plotting log K_1 value versus atomic radius of the metal straight lines are obtained for orotic acid and its *o*-hydroxy arylazo complexes where pK_1 are decreased as the atomic number increased. The effect of substituents from the Hammett constant view point (σ) on the stability constants of the complexes is investigated. Straight line is obtained in copper and nickel complexes derived from -H, *p*-Br and *m*-COOH versus the Hammett constant.

Thermodynamic parameter's of ionization. The behaviour of the free ligands was investigated in the temperature range 27 - 45°, where the ΔG*, ΔH* and ΔS* values are evaluated and collected in Table 4. The pK values for all the compounds except orotic acid and *m*-carboxy phenylazo derivative are decreased with increasing temperature. Also, the pK_1 value for phenylazo compound and its *p*-carboxy derivative is temperature independent. For all compounds, for one and the same temperature, $pK_n > pK_{n-1}$.. and consequently the ΔG value. The isokinetic temperature relationship for the plot of ΔH versus ΔS based on pK_1, pK_2 and pK_3 values, best fit straight lines are obtained. The slopes of these plots are 0.2, 0.35 and 0.35 respectively. The data point to the greater stabilization of such compounds.

References

1. J. W. Daniel, Toxicol. Appl. Pharmacol., **4**, 572 (1962).
2. J. Leberman, A. Kornberg and E. S. Simms, J. Biol. Chem., **215**, 403 (1955).
3. A. Lehninger, *Biochemistry* (Worths Publishers Inc. New York, 1970), pp. 661.
4. D. D., Genchev, Dokl. Boly. Akad. Nauk, **23**, 435 (1970).
5. J. Victor, L.B. Greenberg and D. L. Sloan, J. Biol. Chem. **254**, 2647 (1979).
6. T. Pirmex and C. Jean, Appl., **806**, 8 (1988); C. A., **110**, 236959 K (1989).
7. A. I. Vogel., *A Textbook of Practical Organic Chemistry* (Longmans, London, 1962), 3rd ed.
8. G. Schwarzenbach, *Complexometric Titration* (Methuen Co., London, 1957).
9. L. G. van Uitert and C. G. Hass, J. Chem. Soc., **75**, 452 (1955).
10. R. M. Issa, J. Chem. U.A.R., **14**, 113 (1971).
11. J. C. Colleter, Ann. Chim., **5**, 415 (1960).
12. A. Muka, M. Oravico, J. Analyt. Chim. Acta, **45**, 534 (1969).
13. M. S. Masoud and F. M. El-Zawaway, Talanta, **27**, 766 (1980).
14. H. Irving and H. Rossotti, J. Chem. Soc., 2904 (1954), 3397 (1953).
15. S. Charberk and A. E. Martell, J. Am. Chem. Soc., **74**, 5052 (1952).
16. P. Castan, S. Wimmer, E. R. Colacio and L. A. Beauchamp, J. Inorg. Biochem., **38**, 225 (1990); C. A., **113**, 34415 f (1990).
17. L. F. Wimmer, P. Castan, S. Wimmer and Jonson, Anticancer Res., **9**, 791 (1989); C. A., **111**, 146294 F (1989) (Fr.).
18. F. Guay, A. L. Beauchamp. C. Gilbert and R. Savoie, Can. J. Spectrosc., **28**, 13 (1983).
19. S. J. Yeh and H. H. Jaffe, J. Am. Chem. Soc., **18**, 3283 (1959).
20. M. T. Saks and H. H. Jaffe, J. Am. Chem. Soc., **86**, 2209 (1964).

21. G. Cilents, J. Org. Chem., **24**, 2015 (1959).
22. H. H. Jaffe, Science, **118**, 246 (1953).
23. D. Shugar and J. F. Jack, Biochim. Biophys. Acta, **9**, 199 (1952).
24. D. J. Garter and S. P. Clavdia, Biochim. Biophys. Acta, 214 (1952).
25. A. A. Hasanein, M. S. Masoud and A. M. Heiba, J. Chem. Soc. Pak., **9**, 199 (1987).
26. M. S. Masoud, A. Akelah and S. S. Kandil, Ind. J. Chem., **24A**, 855 (1985).
27. J. G. Jones, J. B. Pode, J. C. Tomkinson and R. J. Williams, J. Chem. Soc., 2001 (1958).