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A semiempirical MO study of tautomerism and the electronic structure of barbituric acid

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Abstract. The electronic structure of barbituric acid has been investigated, keeping in mind the possibility of tautomerism. It has been found that the triketo form is the most stable, followed by the 4-hydroxy tautomer. The difference in their stabilities decreases on substitution at C_5. Substituents that allow a greater degree of delocalization with the ring system (nitro, bromo, thiol) stabilize the 4-hydroxy tautomer to a greater extent since it is planar. The AM1 method is found to be the best suited for studying the electronic structure of barbituric acid, as it gives the best agreement with the experimental geometries. Of the other two methods investigated, MNDO gives erroneous results for relative energies, while PM3 gives unsatisfactory geometries. The stabilities of the charged species resulting from deprotonation, and the radicals resulting from the removal of a hydrogen atom by heat treatment or irradiation with gamma rays, have also been investigated. Their electronic structures are also discussed.

Keywords. Barbituric acid; tautomerism; substituent effects; AM1, MNDO, PM3 calculations; anions; radicals.

1. Introduction

The possibility of tautomerism in pyrimidine derivatives has been the subject of much speculation. Research on various facets of tautomerism continues unabated. Both experimental and theoretical studies (*ab initio* and semiempirical) have been recently reported[1–8]. In particular, the determination of electronic properties of the principal tautomers is of fundamental importance in understanding processes related to molecular biology and pharmacology. Derivatives of barbituric acid are perhaps the most widely used pyrimidines in medicine[9].

It is known that, in the solid state, most pyrimidine derivatives occur in the keto, rather than the enol, configuration. Barbituric acid is interesting in the sense that it can exhibit two kinds of tautomerism-transfer of either the imine hydrogen or a methylene hydrogen to a keto oxygen. The trioxo form of barbituric acid in crystals and aqueous solutions is well established[10,11]. It also exists in the same form in nonpolar solvents since the PMR spectrum in DMSO solvent shows the presence of methylenic protons and the absence of lines corresponding to the protons bonded to unsaturated carbon atoms. Also, on addition of D_2O to barbituric acid solutions in DMSO, the methylenic signal disappears due to the exchange with deuterium. This tendency to exchange is a well-known property of β-dicarbonyl compounds.

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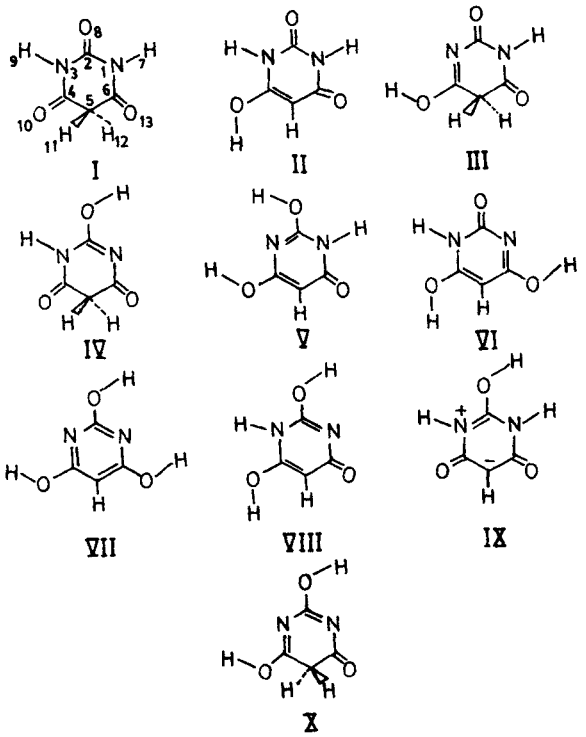

Figure 1. Structures of barbituric acid tautomers.

Barbituric acid can be considered, therefore, as a model system for structures containing a methylenic group at C_5. Although the predominance of the trioxo form (form **I**, see figure 1) is well established, it is usually represented as 2,4,6-trihydroxypyrimidine (form **VII**). Because of the large number of tautomeric forms possible, it is interesting to examine their relative stabilities.

In our studies on barbiturates, we had found that, although the MNDO method is considered unsuitable for studying the relative stabilities of tautomeric systems, it seems to be adequate for investigating the electronic structures and geometries of different tautomeric forms, and its results are consistent with all known experimental facts regarding their reactivity [12] in the case of alloxan.

In this work, we have investigated tautomerism in barbituric acid by the three methods, MNDO [13], AM1 [14] and PM3 [15]. Although an AM1 study is reported [16], the authors did not take all possible tautomeric forms into consideration, nor did they investigate the proper rotamers of the tautomers studied.

2. Computational details

The MOPAC 6.12 program system [17] was used for the present calculations. All structures were completely optimized with respect to the geometry without any conformational or symmetry restrictions. The keywords PRECISE and GNORM=0·0 were used for all geometry optimizations. This ensured that a mean gradient value lower than 0·01 kcal/mol/Å was reached.

3. Results and discussion

A total of ten tautomeric forms (figure 1) are possible, i.e., the triketo form (**I**), the 4-hydroxy forms (**II** and **III**), the 2-hydroxy form (**IV**), the 2,4-dihydroxy forms (**V**, **VIII**, and **X**), the 4,6-dihydroxy form (**VI**), the 2,4,6-trihydroxy form (**VII**), and the zwitterion (**IX**). Combined with the fact that each tautomer may have different rotamers, a total of more than thirty systems were studied.

3.1 *Relative tautomer energies*

The various tautomeric structures (**I-X**) are depicted in figure 1 and the heats of formation are given in table 1. A number of rotamers is possible for all the tautomers, but, in figure 1, only the most stable rotamer for each tautomer is depicted.

In this case, however, the MNDO method gives results at total variance with the AM1 and PM3 calculations. According to the MNDO calculations, the trihydroxy form (**VII**) should be the most favoured, which is against all experimental evidence from X-ray [10,18], ^{14}N-NQR [19], as well as NMR investigations in anhydrous DMSO [20,21]. This reflects the general tendency of the MNDO method to overestimate the stability of fully conjugated aromatic rings.

The other two methods (AM1 and PM3) agree on the stability orders (**I** > **II** > **III** > **IV**), and that the **VIII**, **IX** and **X** tautomers (see figure 1) are the least stable. There are slight differences in the stability orders for the other tautomers, but these differences are insignificant, as these tautomers all have similar energies. It may be noted that PM3 predicts greater stability for the fully conjugated form (**VII**), as compared with the AM1 results. This reflects the tendency of AM1 to disfavour hydroxy forms. As expected, the stable tautomers all involve the migration of a single proton from either the methylene carbon or a nitrogen. The stability of **II** over **III** shows that the migration of a methylene hydrogen is preferred due to the resulting stable structure, and the relative instability of **IV** over **III** indicates that the migration of the imine hydrogen to O_8 (see figure 1) leads to instability.

For the trioxo form, the calculated dipole moment from MNDO (see table 1) is in better agreement with the experimental value of 1.04 Debyes [22] than either the AM1 or

Table 1. Heats of formation (kcal/mol) and dipole moments (Debyes) of barbituric acid tautomers.

	$-\Delta H_f$			μ		
No.	MNDO	AM1	PM3	MNDO	AM1	PM3
I	118·7	107·8	124·0	0·98	0·68	0·81
II	115·1	96·4	116·0	4·14	3·91	4·03
III	115·6	92·3	112·5	3·46	3·42	3·15
IV	112·7	88·2	110·2	2·70	2·84	2·68
V	121·9	87·6	109·8	4·15	4·29	3·96
VI	115·4	85·0	107·8	5·13	4·96	5·11
VII	128·2	83·0	109·9	1·28	1·29	1·20
VIII	106·9	75·5	100·8	5·21	5·18	5·21
IX	91·9	74·1	92·7	7·83	7·88	7·87
X	110·4	72·8	100·2	3·47	3·35	3·34

Table 2. Optimized geometries of barbituric acid (I, C_{2v}).

Bond Parameter[a]	MNDO	AM1	PM3	Expt.[b]
C_2N_1	1·409	1·406	1·424	1·365
C_4N_3	1·414	1·394	1·421	1·390
C_5C_4	1·525	1·508	1·508	1·490
H_7N_1	1·008	0·999	1·000	—
O_8C_2	1·226	1·246	1·221	1·229
$O_{10}C_4$	1·225	1·240	1·219	1·202
$H_{11}C_5$	1·116	1·126	1·112	—
$N_3C_2N_1$	116·0	118·8	118·9	115·1
$C_4N_3C_2$	126·1	123·5	122·6	124·8
$C_5C_4N_3$	117·8	118·9	119·9	119·8
$C_6C_5C_4$	116·3	116·4	116·0	114·0
$H_7N_1C_2$	116·1	116·9	117·8	—
$O_8C_2N_3$	122·0	120·6	120·5	122·6
$O_{10}C_4N_3$	118·0	119·3	116·0	118·1
$H_{11}C_5C_4$	108·3	108·2	108·8	—
$O_{13}C_6C_5$	124·2	121·8	124·1	—
$H_{11}C_5C_4N_3$	122·2	122·0	123·1	—

[a]See figure 1; [b]From Jeffrey *et al* 1961

PM3 values. The large variations in dipole moments suggest that some of the less likely tautomers in the vapour phase may get stabilized in aqueous solution. Accordingly, we calculated the energies of interaction with water for all the tautomers and rotamers to see if any differences in the stability orders arise on solvation. The reaction field continuum model[23] was used for this purpose. The water-tautomer interaction energies are found to be smaller than the differences in stabilities, and no change in the order of stability of the tautomers occurs on solvation with water, although the importance of the hydroxy form (**II**) increases, due to its higher dipole moment.

3.2 *Geometries*

Table 2 gives the optimized geometries for the triketo tautomer of barbituric acid, as determined by the three methods, as well as the experimental geometry for the solid state[10,18,24–26]. The structures are essentially planar. The agreement between the calculated and the experimental geometries is, however, not very good. The reason may be the significant effect on the bond parameters due to hydrogen bonding in the solid state[26] which leads to asymmetry in the geometry. The deviation from experiment is least for AM1.

A point to note is that the PM3 values for the optimized C–N bond distances are much larger than the experimental values and those calculated by other methods (see table 2). There are only slight deviations from planarity and the triketo form (**I**, see figure 1) may be considered to have C_{2v} symmetry (table 2), while the other structures have C_s symmetry.

From the foregoing discussion on the relative tautomer energies and geometries, it is evident that the MNDO relative energies are not in accord with other calculations and experimental results, while the PM3 geometries are incorrect. Therefore, only the AM1 calculations are in relative agreement with experimental observations, although, they

too, tend to overestimate the stability of the trioxo form. To confirm the suitability of AM1 in predicting other known properties of barbituric acid, it was used to investigate these.

3.3 *Frontier orbitals*

The orbital compositions of the highest occupied molecular orbitals (HOMOs) and lowest unoccupied molecular orbitals (LUMOs) were analyzed. It was found that, while the HOMO of the triketo form of barbituric acid is the out-of-plane orbital (b_1) involving N_1, O_8 and N_3 (i.e., the upper part of the ring, see figure 1), the LUMO (b_1) is the carbonyl (C_2=O_8, C_4=O_{10} and C_6=O_{13}) π^* orbital, involving only the lower part of the ring. The orbital energies are, respectively, $-11{\cdot}629$ eV and $-0{\cdot}357$ eV. In fact, the three highest occupied molecular orbitals of barbituric acid are accidentally nearly degenerate. The lowest of these (HOMO-2 (a_2), orbital energy $= -11{\cdot}680$ eV) is an out-of-plane nitrogen nonbonding orbital, with overlaps from the oxygens O_{10} and O_{13} (figure 1). The next orbital (HOMO-1 (b_2), orbital energy $= -11{\cdot}633$ eV) is an in-plane oxygen nonbonding orbital.

Enolization at the 4-position (**II**) results in the HOMO becoming a delocalized π MO (a") comprising the $2p_z$ orbitals of the $N_3C_4C_5$ moiety, with the greatest contribution from C_5 (51%), while the LUMO consists mainly of C_4 (49%) and C_5 (19%). Therefore, in the keto form, the HOMOs involve only the carbonyl oxygens and the nitrogens, with very little contribution from the ring carbons, and the LUMO also comprises the carbonyl antibonding orbitals. However, in the enol form, both the HOMO and LUMO are localized in the ring, with very little involvement of the carbonyl oxygens. The ionization potential also decreases substantially to 9·902 eV.

3.4 *Effect of substitution*

Introduction of substituents at the 5-position results in an increase in the importance of the hydroxy form because the substituent comes into the plane of the ring and, consequently, the greater delocalization of charge stabilizes the 4-hydroxy system; the results have been explained by inductive effects[27]. The effect of monosubstitution at the 5-position was investigated at the AM1 level and it was found that the keto-enol energy separation decreases from the value of 11·4 kcal/mol in the unsubstituted compound. The difference varies in the order $-H > -CH_3 \approx -C_2H_5 > -OH > -F > -Cl > -I > -Br > -NH_2 > -SH > -NO_2$. For the latter two substituents, the difference is negative, pointing to the greater stability of the 4-hydroxy form with respect to the triketo form. For $-NO_2$ (dilituric acid), this is in accord with experimental observations in the crystalline state as shown by X-ray analysis[28]. Infrared[29] and ultraviolet investigations[30], dipole moment measurements[31] and ^{14}N-NQR data[32] also support this conclusion. For –OH (dialuric acid), however, while experimentally the 4-hydroxy form is observed to be more stable in the crystalline state[33,34], at the AM1 level, the keto form is more stable. It is gratifying to note, however, that a study of the line width and chemical shift versus temperature for –OH and –NH signals for barbituric acid and dialuric acid ruled out any appreciable contribution from enolic forms in both cases, signifying that, in solution, the triketo form is the only one making any contribution[21]. The calculated order of keto-enol separation is in general agreement with the substituent effects on the stability of enols and enolates relative to their oxo forms in a recent G2(MP2) *ab initio* study[35] on

acetaldehyde derivatives. The author observed that, for the acetaldehyde system, both σ electron-withdrawing and π-donating substituents favour the oxo form over the enol form, since these substituents ($-NH_2$, $-OH$, $-F$) favour both forms with the oxo form to a greater extent. In the present case, however, there is an additional stabilization of the enol form due to a larger conjugation in this form, since it is planar.

Since the tautomer(**II**) of barbituric acid has a higher dipole moment (3·91D) than the triketo form (**I**, 0·68D), the solute-solvent interaction energies were evaluated for both tautomers of substituted barbituric acids to see if the enol form gets enough stabilization in aqueous solution to change the stability over. As before, the reaction field continuum model was used.

Since the dipole moments for the keto forms are very small, very little stabilization occurs in aqueous solution. On the other hand, the dipole moments for the enol forms are much higher, and interaction with the solvent produces greater stabilization. Hence the differences in energies between the two forms decrease on solvation, although the order of relative stability remains essentially unchanged, as the dipole moments for the derivatives are similar ($\sim$ 4 D), except the enol form of dialuric acid, which has a lower dipole moment (2·4 D) than the rest of the enol systems. It has been reported that the gas phase enolization enthalpies for cycloketones are a little lower than those reported from the aqueous solution measurements since the enol tautomers are more sensitive to aqueous solvation than their keto tautomers [6,36,37].

3.5 *Charged species*

Due to its high electron potential and positive electron affinity, barbituric acid readily loses a proton to form the anion. Barbituric acids are often employed to buffer aqueous solutions, especially of biological media, and an accurate knowledge of the acid dissociation constants is useful in work on blood chemistry [38]. Furthermore, there is a relationship between the dissociation constant of a barbituric acid and its ability to produce sedation [39,40].

The resulting anion may also exist in different tautomeric forms. Considering the overwhelming stability of the **I** and **II** tautomers of barbituric acid, we considered only deprotonation from these two forms, which results in three structures (figure 2).

It is again found that the tautomeric structure **II** ($\Delta H_f = -148{\cdot}8$ kcal/mol), which results from deprotonation from the methylene group of the triketo form (**I**) or the hydroxyl group of the tautomer (**II**, see figure 1), is the most stable, while **III** ($\Delta H_f = -120{\cdot}9$ kcal/mol) is the least stable, signifying that deprotonation occurs from the methylene carbon, C_5, and barbituric acid is a carbon acid. The formation of the anion is accompanied by a decrease in all ring distances except the C_4–N_3 and C_6–N_1 bond distances (see figure 1). The C=O bond distances also increase slightly. The bond orders of all the carbonyl bonds, including the remote C_2=O_8 carbonyl bond, reduce to $\sim$ 1·6, signifying the amount of delocalization of the electron. The negative charge gets distributed on C_5 and the nitrogens and oxygens.

The loss of a methylenic proton from substituted barbituric acids must result in greater stability of the resulting anion. McMahon and Kebarle defined the intrinsic gas phase acidities of carbon and nitrogen acids by the expression D(R–H)-EA(R), where D is the dissociation energy of the R–H bond, and EA is the electron affinity of the resulting radical[41]. For barbituric acid, they obtained a value of 16·3 kcal/mol. Our calculated value is 16·4 kcal/mol. Because of the excellent agreement between the

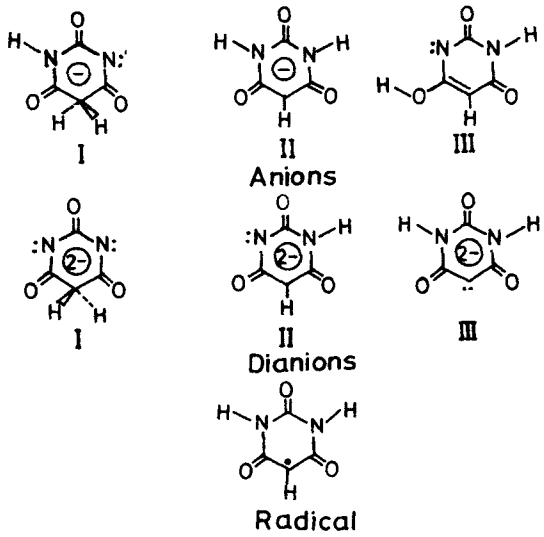

Figure 2. Structures of anions and dianions.

experimental and calculated values, we calculated the corresponding values for various substituents. The values decrease in the order $H > -C_2H_5 > -CH_3 > -NO_2 > -OH > -Cl > -F > -NH_2 > -I > -Br > -SH$, which is the order of increasing gas phase acidities. The experimental solution phase pK_a values of 4·02, 3·69, and 3·39 for $-H$, $-C_2H_5$, and $-CH_3$ [42] are in agreement with this order, although this is against the order expected from a consideration of the usual inductive electron-donating effect of alkyl groups.

Theoretically, three dianions (see figure 2) are possible by the loss of any two protons. The most stable of these has one proton removed from a nitrogen and the other from the methylene group (**II**). The heats of formation of **I**, **II** and **III** are $-63·5$, $-71·7$ and $-15·1$ kcal/mol, respectively. Loss of protons from N_1 and C_5 results in the negative charge getting concentrated on the nitrogens and the oxygens, particularly O_8. Only C_5, among the ring carbon atoms, is negatively charged. The rest are electron deficient, even in the dianion.

3.6 *Radicals*

γ Irradiation of barbituric acid produces a stable radical by the loss of a hydrogen from the C_5 position [43]. The resultant radical has an unpaired electron in the $2p\pi$ orbital of C_5 (73%) which is delocalized over the $2p\pi$ orbitals of O_{10} and O_{13} (10% each). The negative charge on these two oxygens decreases, showing that these two atoms transfer electron density to C_5. The magnitude of the spin density (0·85) is maximum at C_5, in agreement with the experimental value [43] of 0·73, and is also finite at the oxygens of the carbonyl groups at C_4 and C_6 ($-0·31$).

For substituted barbituric acids, it was found that the radical prefers the keto form, even for those cases ($-NO_2$, dilituric acid) for which the parent compound prefers the 4-hydroxy form. In the case of dialuric acid, the hydroxyl group is perpendicular to the plane of the ring in the anion, but becomes planar in the radical. In uramil, while,

in the 4-hydroxy tautomer and the anion, the $-NH_2$ group is perpendicular to the ring, in the radical, it remains in the ring plane. The opposite is the case for dilituric acid, where the substituent $-NO_2$ group is perpendicular to the ring plane in only the radical.

4. Conclusions

Our calculations on barbituric acid have shown that, of the various possible tautomeric structures, only the triketo form and the 4-hydroxy form are important. Substituents decrease the separation in their energies, making the hydroxy form more important for substituents which allow greater delocalization of charge in the 4-hydroxy planar systems. The AM1 method is best suited to study the electronic structure and properties of barbituric acid. Most of the experimental results concerning its properties are correctly reproduced by this method. The calculated properties for the charged species and radicals are also in satisfactory agreement with experiment.

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References

1. Wu D-H and Ho J-J 1998 *J. Phys. Chem. A* **102** 3582
2. Gorb L and Leszczynski J 1998 *J. Am. Chem. Soc.* **120** 5024
3. Leszczynski J 1998 *J. Phys. Chem. A* **102** 2357
4. Rubin Y V, Morozov Y, Venkateswarlu D and Leszczynski J 1998 *J. Phys. Chem. A* **102** 2194
5. Luyten I, Pankiewicz K W, Watanabe K A and Chattopadhyaya J 1998 *J. Org. Chem.* 1033
6. Zhang X-M, Malick D and Petersson GA 1998 *J. Org. Chem.* **63** 5314
7. Das S and Dogra S K 1998 *J. Chem. Soc., Faraday Trans.* **94** 139
8. Broo A 1998 *J. Phys. Chem. A* **102** 526
9. Andrews E, Bennett B T, Clark J D, Houpt K A, Pascoe P J, Robinson G W and Boyce J R 1993 *J. Am. Vet. Med. Assoc.* **202** 229
10. Jeffrey G A, Ghose S and Warwicker J O 1961 *Acta Crystallogr.* **14** 881
11. Katritzky A R and Lagowski J M 1963 *Adv. Heterocycl. Chem.* **1** 375
12. Kakkar R and Sarma B K 1997 *Indian J. Chem.* **B36** 1148
13. Dewar M J S and Thiel W 1977 *J. Am. Chem. Soc.* **99** 4899
14. Dewar M J S, Zoebisch E G, Healy E F and Stewart J J P 1985 *J. Am. Chem. Soc.* **107** 3902
15. Stewart J J P 1989 *J. Comput. Chem.* **10** 209
16. Millefiori S and Millefiori A 1989 *J. Heterocycl. Chem.* **26** 639
17. Stewart J J P 1990 *Frank J. Seiler Res. Lab., U.S. Air Force Academy, COLO, SPGS CO80840; MOPAC 6.12, QCMP Program 137, Bloomington, IN.*
18. Bolton W 1963a *Acta Crystallogr.* **16** 166
19. Maruizumi T, Hiyama Y and Niki E 1980 *Bull. Chem. Soc. Jpn.* **53** 1443
20. Nevill G A and Cook D 1969 *Can. J. Chem.* **47** 743
21. Glasel G A 1969 *Org. Magn. Reson.* **1** 481
22. Soundarajan S 1958 *Trans. Faraday Soc.* **54** 1147
23. Rein R 1971 *Int. J. Quant. Chem., Symp.* **4** 341
24. Voet D 1972 *J. Am. Chem. Soc.* **94** 8213
25. Bideau J P, Huong P V and Toure S 1976 *Acta Crystallogr.* **B32** 481
26. Craven B M, Cusatis C, Gartland G L and Vizzini 1973 *J. Mol. Struct.* **16** 331
27. Slesarev W I and Ivin B A 1974 *Zh. Org. Khim.* **10** 113
28. Bolton W 1963b *Acta Crystallogr.* **16** 950
29. Mihai F and Nutiu R 1968 *Rev. Roum. Chim.* **13** 39

30. Simon Z, Mihai F and Nutiu R 1968 *Rev. Roum. Chim.* **13** 147
31. Nutiu R, Kurunczi L and Simon Z 1969 *Rev. Roum. Chim.* **14** 1435
32. Subbarao S N and Bray P J 1977 *J. Chem. Phys.* **67** 1085
33. Bolton W 1965 *Acta Crystallogr.* **19** 1051
34. Craven B M and Sabine T M 1969 *Acta Crystallogr.* **B25** 1970
35. Rosenberg R E 1998 *J. Org. Chem.* **63** 5562
36. Pollack S K and Hehre W J 1977 *J. Am. Chem. Soc.* **99** 4845
37. Chiang Y, Kresge A J and Schepp N P 1989 *J. Am. Chem. Soc.* **111** 3977
38. Manov G G, Schuette K E and Kirk F S 1952 *J. Res. Natl. Bur. Stand.* **48** 84
39. Klimesch K 1933 *Arch. Exp. Path. Pharmak.* **172** 10
40. Burger A 1963 *Medicinal chemistry* (New York: Interscience) pp 363
41. McMahon T B and Kebarle P 1976 *J. Am. Chem. Soc.* **98** 3399
42. Koffer H 1974 *J. Chem. Soc., Perkin II* 1428
43. Bernhard W and Snipes W 1966 *J. Chem. Phys.* **44** 2817