Electronic structures of melatonin and related compounds studied by photoelectron spectroscopy

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Abstract

Melatonin is a hormone structurally regarded as being composed of a 5-methoxyindole group and an *N*-ethylacetamide group; its various physiological activities have attracted a great deal of attention recently. The gas phase He(I) photoelectron spectra of melatonin (M) and its related compounds including *N*-acetylserotonin have been studied with the aid of molecular orbital calculations. The first photoelectron spectral band group of compound M is ascribed to ionizations from the two π orbitals localized on the methoxyindole group. The second band group is quite complicated and is regarded as being composed of several bands. The lower energy part of the second band group is ascribed to the three orbitals relevant to the third highest occupied π orbital of 5-methoxyindole and the highest occupied π and the $n_{C=O}$ orbitals of *N*-ethylacetamide. The interactions among the three orbitals have been found to operate on the basis of the molecular orbital calculations; these interactions depend strongly on the conformations. The high energy end of the second band group is relevant to the π orbital mainly localized on the 5-methoxyindole group and is ascribed to the fourth highest occupied π orbital of 5-methoxyindole.
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Keywords: He(I) photoelectron spectra; Melatonin; *N*-Acetylserotonin

1. Introduction

Melatonin (M; *N*-acetyl-5-methoxytryptamine) is one of the tryptophan metabolites and a hormone secreted from the pineal gland. This compound was isolated from many thousands of beef pineal glands and synthesized by the American dermatologist Aaron Lerner in 1958, to identify an amphibian skin-lightening hormone [1]. This compound provides life-support in living bodies. Recently, the various physiological activities of melatonin have attracted a great deal of attention. In particular, its function as a sleeping drug has received a lot of publicity in the mass media and the compound is now easily available in drugstores in some countries. However, the mechanisms of its activities are still unknown.

The gas phase ultraviolet photoelectron spectrum of melatonin has already been measured [2]. However, the former study has not discussed spectral band assignment except to state that the first band group is associated with the two highest occupied π orbitals which are related to the π_1 and π_2 bands of indole (I). In this paper, the gas phase He(I) photoelectron spectra of melatonin and its related compounds have been measured and investigated in order

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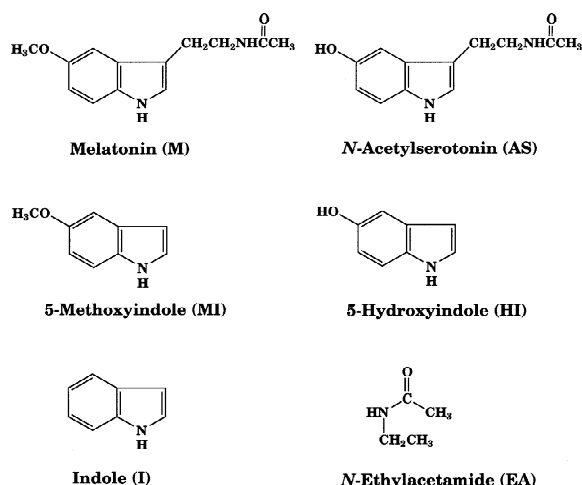*E-mail address:* marik@hc.cc.keio.ac.jp (M. Kubota).

Fig. 1. Structural formulae of melatonin (M) and related compounds AS, MI, HI, I and EA.

to clarify the detailed electronic structure of melatonin which is fundamentally important for the future development of melatonin chemistry. The compounds treated in this study are as follows: melatonin (M), *N*-acetylserotonin (AS; *N*-acetyl-5-hydroxytryptamine), 5-methoxyindole (MI), 5-hydroxyindole (HI), indole (I) and *N*-ethylacetamide (EA). Roughly speaking, compound M is regarded as being composed of compounds MI and EA. Compound AS is structurally very close to compound M and is regarded as being composed of compounds HI and EA. The structural formulae of these compounds are shown in Fig. 1.

2. Experimental

All the compounds measured here were purchased from Tokyo Chemical Industry and were used without further purification.

The gas phase ultraviolet photoelectron spectra were measured with the He(I) photoelectron spectrometer described formerly [3–5]. In this work, NAIG E-562 and E-564 process memories were used to accumulate the signals and the accumulated spectral data were transferred to a personal computer, NEC 98XA, and the data were processed. The spectra remained constant in shape during the measurements, indicating that decomposition did not

occur. The estimated accuracy of the energy scales is ±0.01 eV. Nitrogen gas was used as the internal standard for the calibration of the energy scale. The sample inlet and target chamber systems were heated and their temperatures were maintained constant to ±4 °C (on average) during the measurements by circulating hot silicon oil. The sample gas temperatures during the spectral measurements for compounds M, AS, MI, HI, I and EA were 139, 166, 129, 127, 42 and 25 °C, respectively.

3. Computational

In order to assign the photoelectron spectral bands, molecular orbital calculations have been carried out. Two kinds of semiempirical molecular orbital methods, i.e. AM1 [6] and PM3 [7,8] methods, were carried out by SPARTAN Ver. 4.1 [9]. We have performed ab initio calculations also using the STO-3G, 3-21G and 6-31G* basis sets, respectively, by this program package. For all the methods and for all the compounds the structural refinements have been performed without any restrictions. For compounds M and AS, we have also performed calculations for some conformations.

4. Results and discussion

4.1. Photoelectron spectra

The He(I) photoelectron spectra measured for compounds M, AS, MI, HI, I and EA are shown in Fig. 2, and the vertical ionization energy (E_{iv}) values of these compounds are summarized in Table 1. The numerical values in the parentheses indicate the apparent band maxima throughout this article.

Some apparent features of the photoelectron spectra of these compounds are as follows.

In compound I the first band group shows two apparent band maxima at 7.9 and 8.4 eV in the energy region 7.7–9.0 eV. The band observed at 9.82 eV is sharp and well separated from the other bands. The following bands are seen at 11.1 and 11.6 eV.

In compounds HI and MI, the first band groups appear in the energy region 7.2–9.0 eV. In compound HI, the two bands exist in the energy region 9.4–
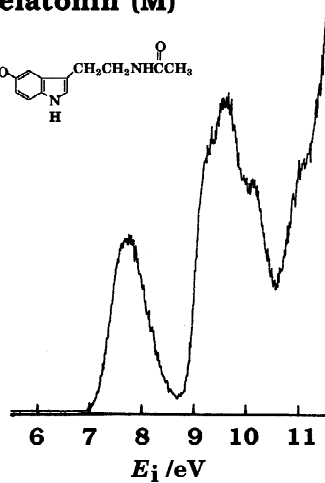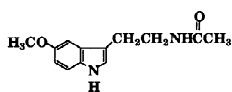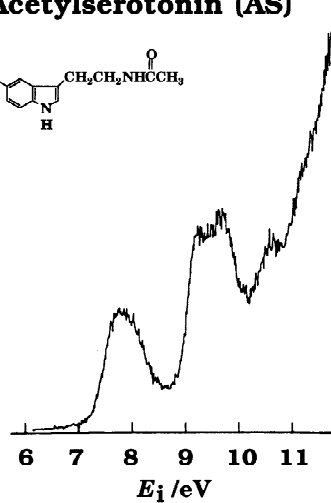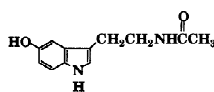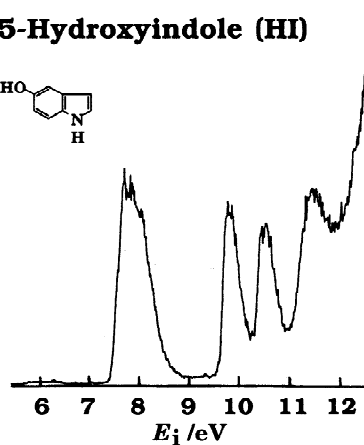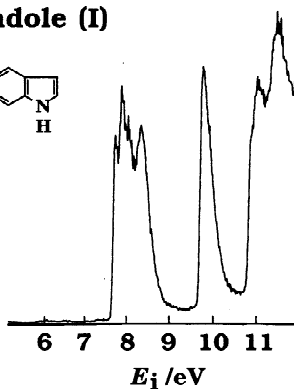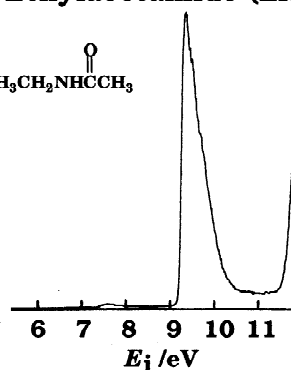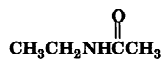
Melatonin (M)

N-Acetylserotonin (AS)

5-Methoxyindole (MI)
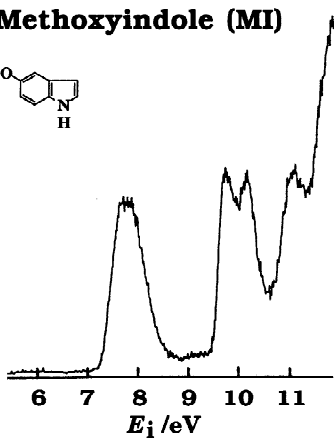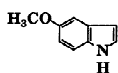
5-Hydroxyindole (HI)

Indole (I)

N-Ethylacetamide (EA)

Fig. 2. Photoelectron spectra of compounds M, AS, MI, HI, I and EA.

Table 1
Vertical ionization energy (E_{iv}) values of compounds M, AS, MI, HI, I and EA

Compound	E_{iv}/eV					
	1st	2nd	3rd	4th	5th	6th
M	(7.76)	(7.76)	(9.33)	(9.66)	(9.66)	(10.12)
AS	(7.79)	(7.93)	(9.26)	(9.65)	(9.65)	(10.60)
MI	(7.73)	(7.90)	(9.76)	(10.19)	(11.14)	
HI	(7.72)	(7.88)	9.79	10.55	(11.47)	
I	(7.93)	(8.38)	9.82	(11.08)	(11.55)	
EA	(9.41)	(9.41)				

The numerical values in the parentheses indicate the apparent band maxima throughout this article.

11.0 eV. On the other hand, in compound MI, the second band group shows two apparent bands in the energy region 9.3–10.6 eV.

In compound EA, the first band group appears at 9.4 eV well separated from the following band group.

In compounds AS and M, the first band groups appear in the energy region 7.0–8.7 eV as compounds HI and MI. Both of the second band groups, however, are quite complicated. In compound AS, the second band group appears in the energy region 8.7–10.2 eV and the third band appears around 10.6 eV. In compound M, the second band group appears in the energy region 8.7–10.5 eV, the high energy end peak of this second band group being observed at 10.1 eV.

A detailed discussion about the photoelectron spectral band assignments of these compounds follows below.

4.2. Assignments of photoelectron spectra

4.2.1. Indole (I)

There have been several investigations of the ultraviolet photoelectron spectra of compound I so far [10–17]. According to the former studies, the first three photoelectron spectral bands of compound I are associated with the four highest occupied π orbitals and the fourth band is associated with the σ orbital which is the fifth highest orbital. Here, we call the first four π orbitals the π_1, π_2, π_3 and π_4 orbitals. The first band group is ascribed to ionizations from the π_1 (I) and π_2 (I) orbitals. The ionization energies of the π_1 (I) and π_2 (I) orbitals are 7.9 and 8.4 eV, respectively. The second band at 9.82 eV is a single band and is ascribed to ionization from the π_3 (I) orbital. The next peak at 11.1 eV is ascribed to ionization from the π_4 (I) orbital. The subsequent peak at 11.6 eV is ascribed to ionization from a σ orbital. The orbital energy values calculated by several molecular orbital methods and the observed vertical ionization energy values for molecule I are summarized in Table 2. The AM1 method predicts the incorrect ordering for the π_4 (I) and σ (I) orbitals. The PM3 method is not bad as the spacings among the first five bands show. The calculated highest occupied molecular orbitals with the PM3 method for compound I are drawn schematically in Fig. 3.

4.2.2. 5-Hydroxyindole (HI) and 5-methoxyindole (MI)

In 5-hydroxyindole (HI), the apparent maxima of the first band group are observed at 7.7 and 7.9 eV and in 5-methoxyindole (MI) at 7.7 and 7.9 eV, respectively: these values are almost identical to each other. Compounds HI and MI are 5-hydroxyl and 5-methoxyl substitution products of compound I, respectively. In both compounds, the π_1 (I) and π_2 (I) bands should be shifted to the lower energy side by the conjugative effects of these substituent groups. Therefore, the first band group of compound HI or MI is also reasonably ascribed to ionizations from the π_1 (HI or MI) and π_2 (HI or MI) orbitals. In compound HI, the second 9.79-eV and third 10.55-eV bands are ascribed to ionizations from the π_3 (HI) and π_4 (HI) orbitals, respectively. In compound MI, the two peaks (9.8 and 10.2 eV) of the second band group correspond to ionizations from the π_3 (MI) and π_4 (MI) orbitals. Here, π_3 (HI or MI) orbital corresponds to the π_3 (I) orbital; the π_4 (HI or MI) orbital is the MO formed by the antibonding type of combination of the n_{OH} or n_{OCH3} orbital,

Table 2
Calculated higher occupied orbital energy (ϵ) values by several molecular orbital methods, the observed vertical ionization energy (E_{iv}) values and the orbital assignments for compounds EA, I, HI and MI

Orbital	E_{iv}, $-\epsilon$/eV					
	Obs.	Calc.				
		AM1	PM3	STO-3G	3-21G	6-31G*
N-Ethylacetamide (EA)						
π_1	(9.41)	10.035	9.676	8.048	10.029	10.335
$n_{C=O}$	(9.41)	10.793	10.924	8.966	10.883	11.169
Indole (I)						
π_1	(7.93)	8.403	8.468	6.152	7.784	7.602
π_2	(8.38)	8.775	8.970	6.516	8.211	8.006
π_3	9.82	10.513	10.451	8.889	10.517	10.283
π_4	(11.08)	12.222	11.871	11.258	12.757	12.537
σ	(11.55)	11.649	12.230	11.324	13.037	12.973
5-Hydroxyindole (HI)						
π_1	(7.72)	8.300	8.330	5.843	7.697	7.538
π_2	(7.88)	8.645	8.865	6.331	7.990	7.729
π_3	9.79	10.599	10.426	8.910	10.600	10.340
π_4	10.55	11.587	11.379	10.240	12.163	12.043
σ	(11.47)	11.745	12.312	11.038	13.018	13.014
5-Methoxyindole (MI)						
π_1	(7.73)	8.218	8.295	5.768	7.564	7.428
π_2	(7.90)	8.593	8.837	6.303	7.924	7.690
π_3	(9.76)	10.521	10.361	8.842	10.516	10.278
π_4	(10.19)	11.224	11.157	9.884	11.793	11.743
σ	(11.14)	11.594	12.151	10.515	12.524	12.681

respectively, with the π_4 (I) orbital. The calculated highest occupied molecular orbitals with the PM3 method for compounds HI and MI are drawn schematically in Figs. 4 and 5, respectively. The orbital energy values calculated by several molecular orbital methods and the observed vertical ionization energy values for molecules HI and MI are given in Table 2. All methods predict the correct ordering of the molecular orbitals, although the spacings among the first five bands are not good, especially so with the ab initio calculations.

4.2.3. N-Ethylacetamide (EA)

The ultraviolet photoelectron spectrum of *N*-methylacetamide has already been measured [18]. On the basis of the previous study on carboxylic acid and their derivatives, the first band group of *N*-ethylacetamide (EA) is safely ascribed to ionizations from the π_1 (EA) and $n_{C=O}$ (EA) orbitals. The

apparent peak of the first band group of compound EA is 9.4 eV. The observed vertical ionization energy values and the calculated orbital energy values for molecule EA are given in Table 2. All the calculations in this work suggest that the π_1 (EA) orbital is higher than the $n_{C=O}$ (EA) orbital, however, in reality the corresponding two bands are extensively overlapping one another.

As for the most stable conformation of molecule EA all the calculations converged to the nearly planar *E* form (in the *Z*, *E*-expression) amide skeleton.

4.2.4. Melatonin (M) and N-acetylserotonin (AS)

As mentioned above, compound M is regarded as being composed of compounds MI and EA and compound AS is regarded as being composed of compounds HI and EA. We have discussed already the observed ionization energy values and the orbital
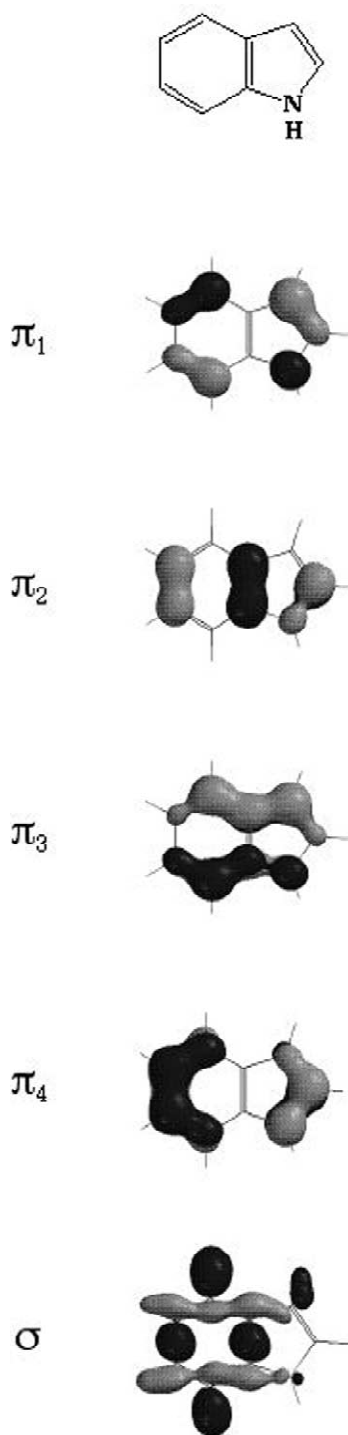
Fig. 3. Schematic representation of the calculated highest occupied molecular orbitals for indole (I).
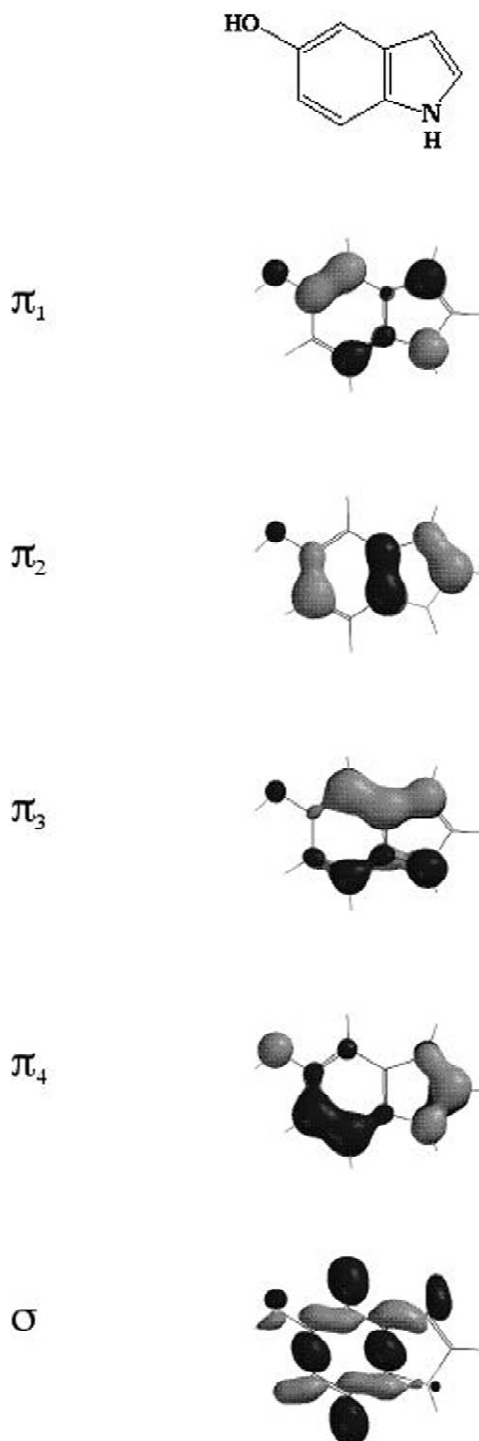
Fig. 4. Schematic representation of the calculated highest occupied molecular orbitals for 5-hydroxyindole (HI).
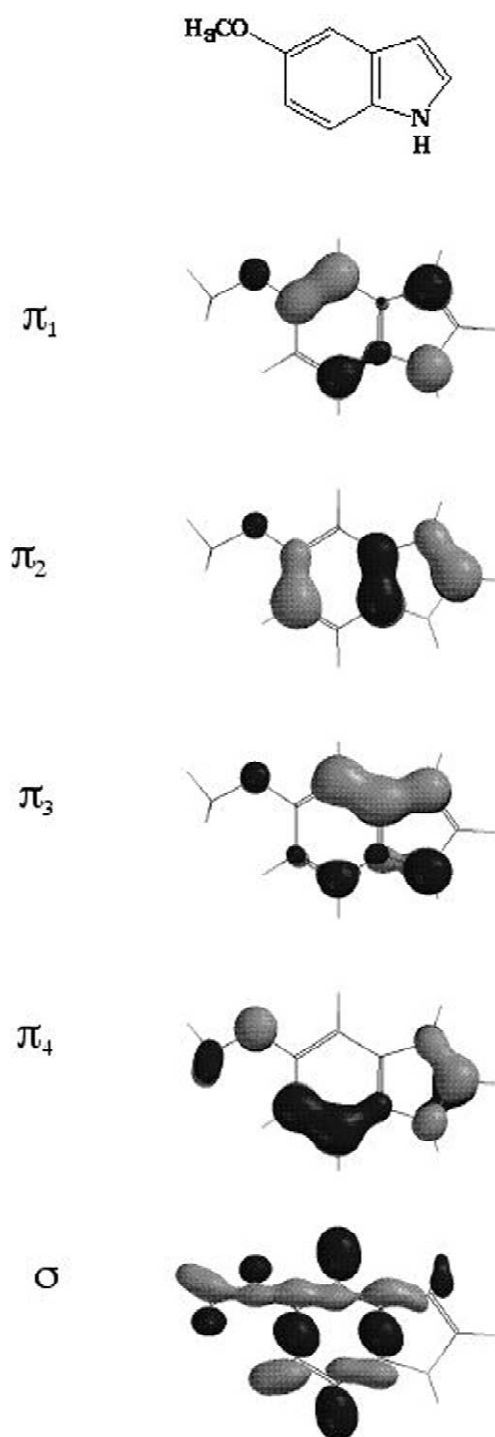
Fig. 5. Schematic representation of the calculated highest occupied molecular orbitals for 5-methoxyindole (MI).

energy values calculated by some molecular orbital calculations for molecules I, MI, HI and EA. On the basis of the discussion given above, we now adopt the PM3 method for molecules M and AS. By the way, the molecular conformations of compounds M and AS in the gaseous phases are still unknown, while MO calculations predict some stable conformations. The orbital energy values calculated by the PM3 method for two typical conformers of molecules M and AS are summarized in Tables 3 and 4, respectively. Here, we refer to the perpendicular type when the indole framework and N-ethylacetamide group are perpendicular to each other and to the parallel type when the indole framework and N-ethylacetamide group are parallel. The observed ionization energy correlation diagram for compounds M, MI and EA are shown in Fig. 6. In this figure, the highest occupied molecular orbitals

Table 3
Calculated higher occupied molecular orbital energy (ϵ) values with the total energy (E_{total}) values by the PM3 method for two typical conformers of compound M

Orbital	$-\epsilon$/eV	
	Perpendicular	Parallel
π_1	8.425	8.516
π_2	8.999	8.951
π_3	9.570	9.761
π_4	10.444	10.569
$n_{C=O}$	10.843	10.915
π_5	11.296	11.284
E_{total}/eV	-2698.636	-2698.766

Table 4
The calculated higher occupied molecular orbital energy (ϵ) values with the total energy (E_{total}) values by the PM3 method for two typical conformers of compound AS

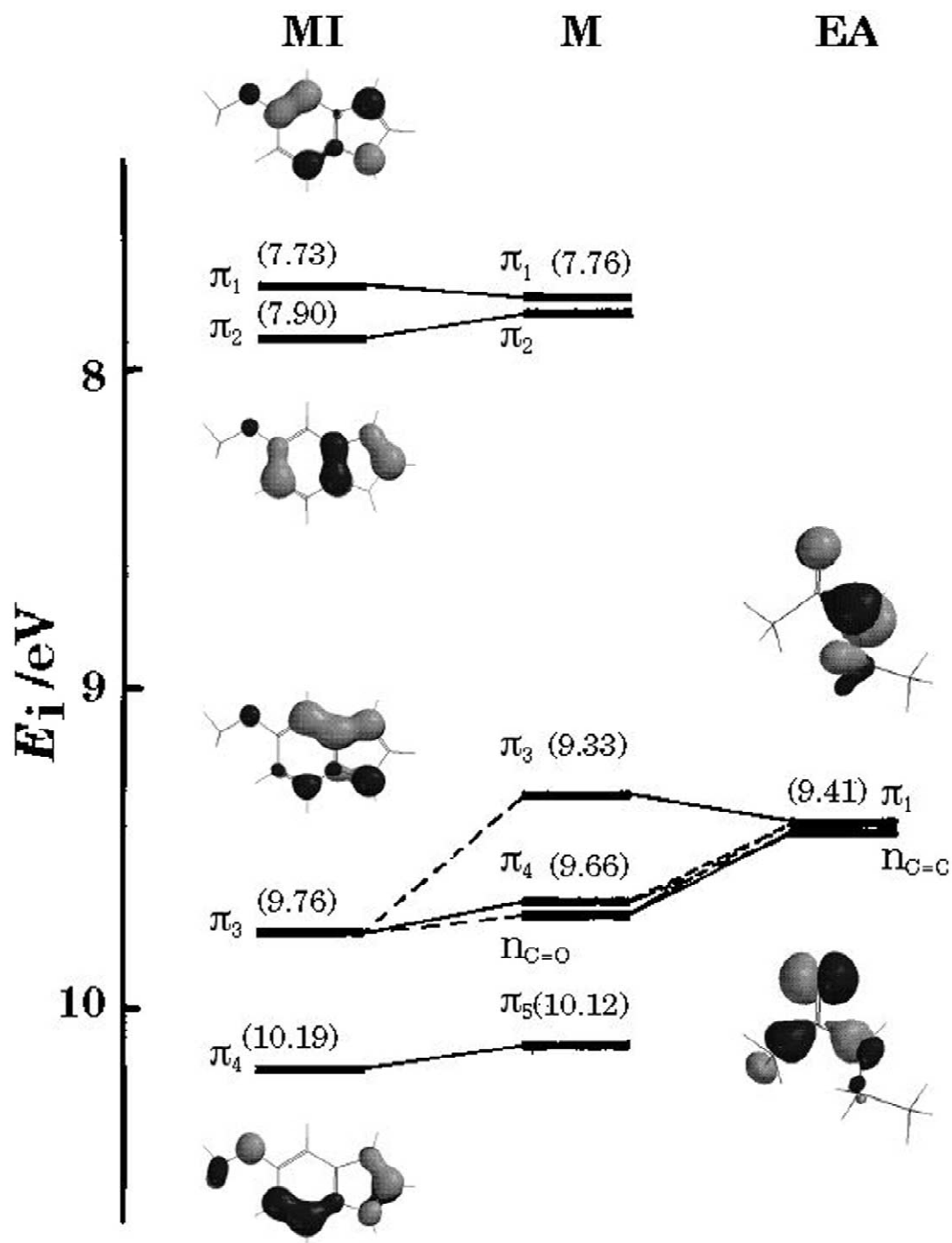| Orbital	$-\epsilon$/eV	
	Perpendicular	Parallel
π_1	8.472	8.540
π_2	9.032	8.994
π_3	9.566	9.783
π_4	10.509	10.616
$n_{C=O}$	10.843	10.930
π_5	11.528	11.535
E_{total}/eV	-2549.648	-2549.754

Fig. 6. Observed ionization energy diagram for 5-methoxyindole (MI), melatonin (M) and *N*-ethylacetamide (EA).
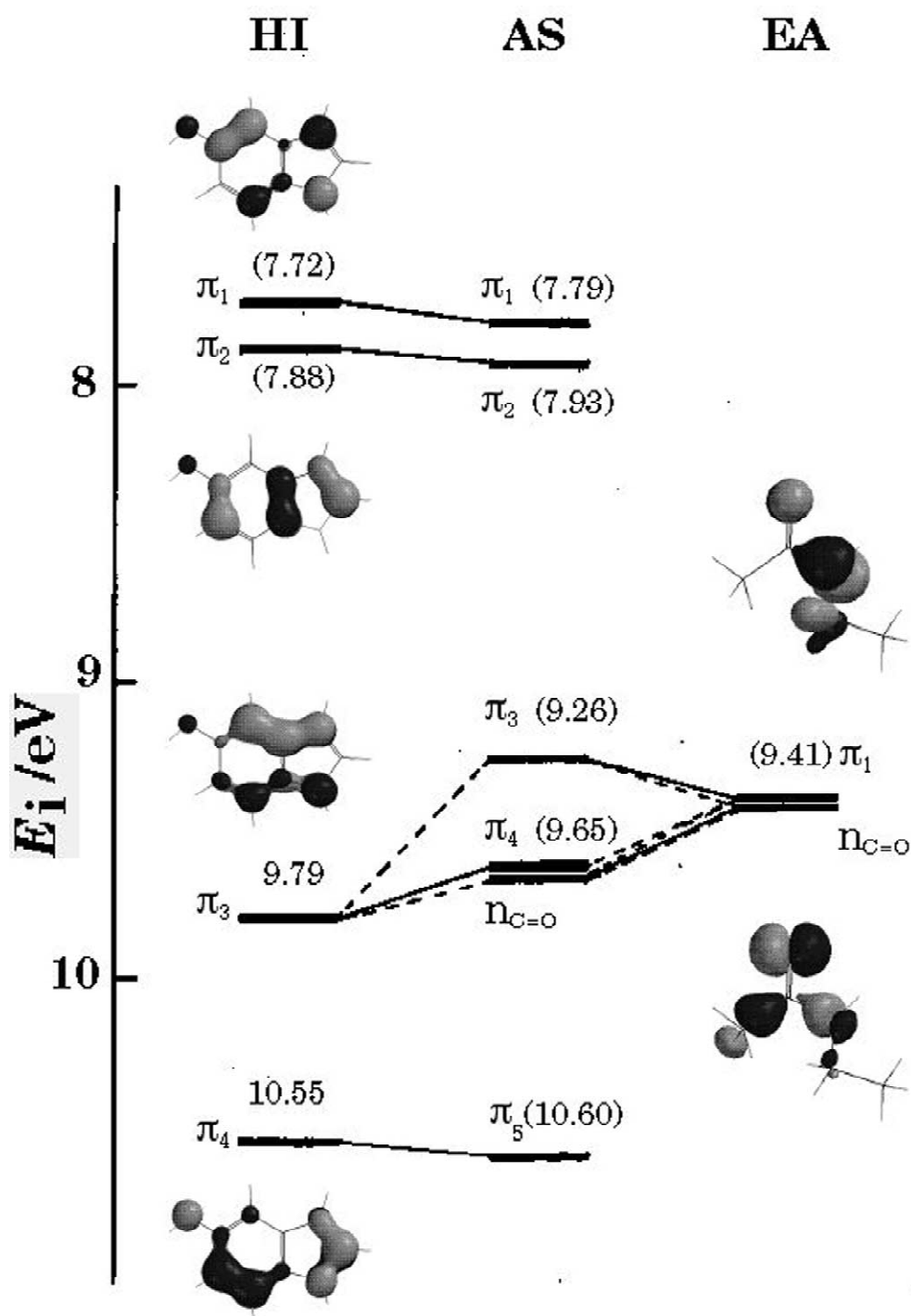
Fig. 7. Observed ionization energy diagram for 5-hydroxyindole (HI), *N*-acetylserotonin (AS) and *N*-ethylacetamide (EA).

calculated by the PM3 method are drawn schematically. Similarly, the energy diagram for compounds AS, HI and EA are also shown in Fig. 7.

In both photoelectron spectra of compounds M and AS, the first band groups appear in the energy region 7.0–8.7 eV as in compounds HI and MI. The

(a)
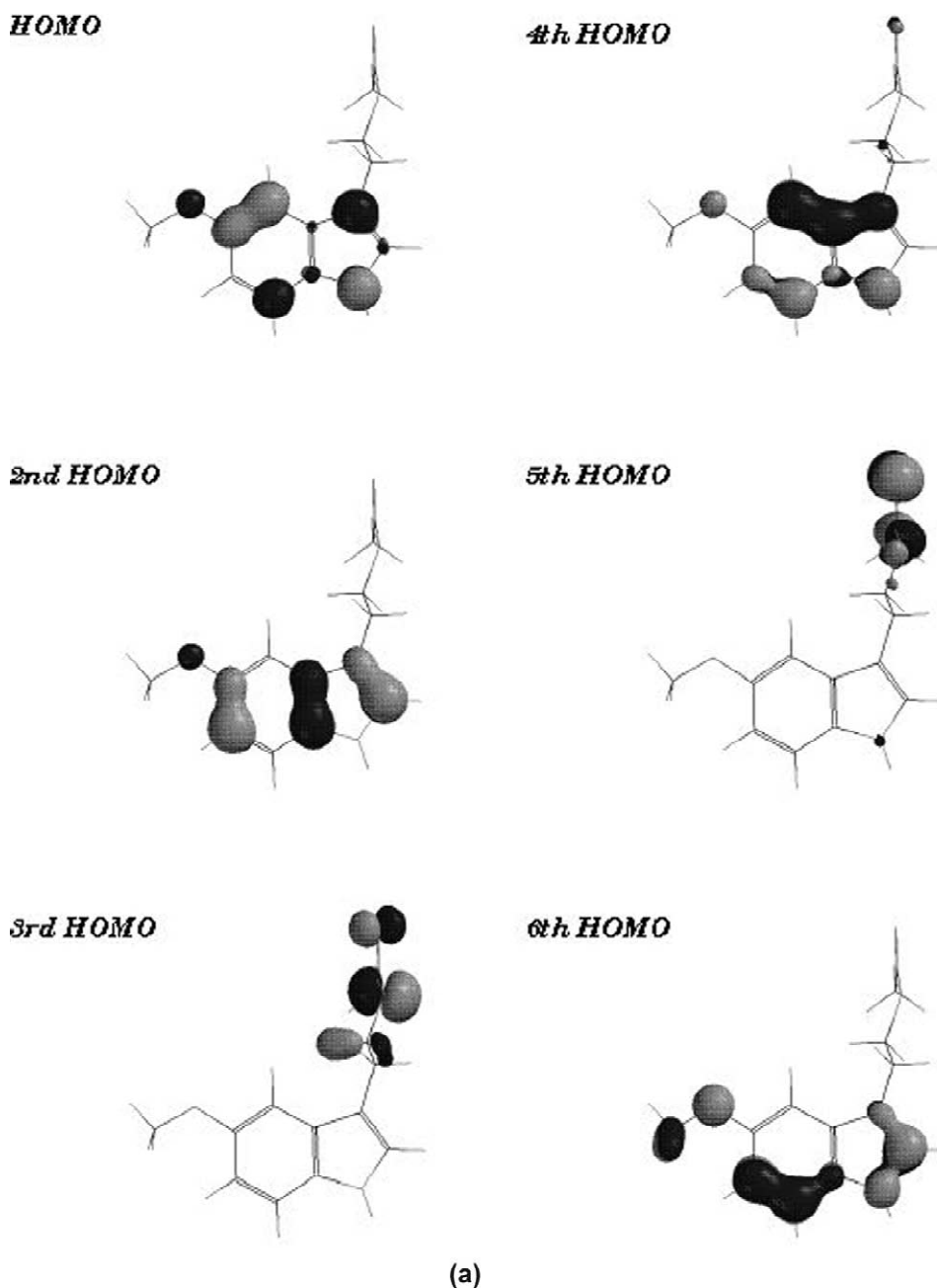
Fig. 8. Schematic representation of the calculated highest occupied molecular orbitals for the two typical conformers of melatonin (M): (a) perpendicular type; (b) parallel type.

first band group of compound M or AS corresponds to the first band group of compound MI or HI, respectively. The molecular orbital calculations also suggest that the π_1 (M or AS) and π_2 (M or AS)

molecular orbitals are quite similar to the π_1 (HI or MI) and π_2 (HI or MI) molecular orbitals.

Both second band groups of compounds M and AS are quite complicated. This is because the π_3

HOMO

2nd HOMO

3rd HOMO

4th HOMO

5th HOMO
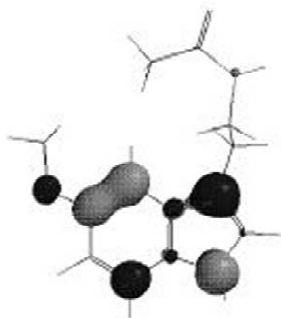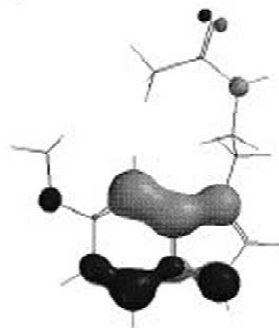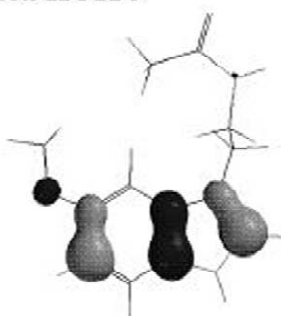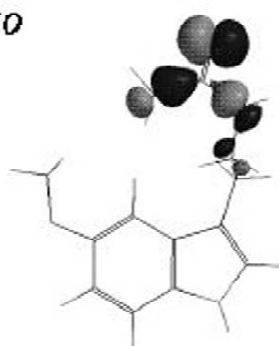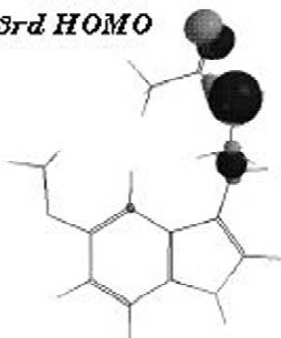
6th HOMO
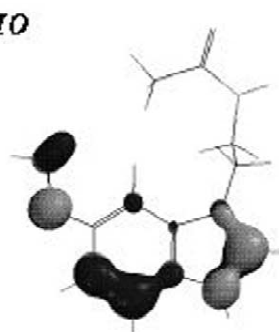
(b)

Fig. 8. (*continued*)

(MI or HI) and π_4 (MI or HI) orbitals are very close in energy to π_1 (EA) and $n_{C=O}$ (EA) orbitals. These orbitals may only nearly degenerate with each other, or may be able to interact. The highest energy peak in the second band group of compound M is observed at 10.1 eV, being very close to the ionization energy value relevant to the π_4 (MI) orbital, 10.2 eV. Similarly, the energy value of the third band
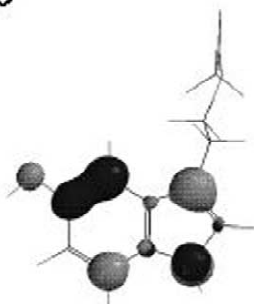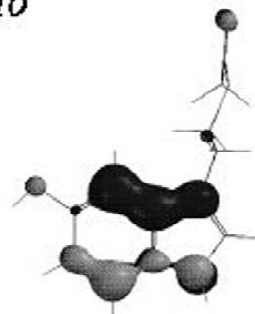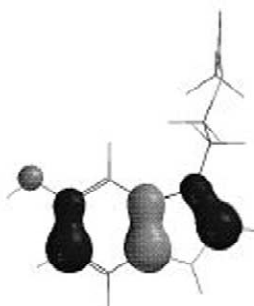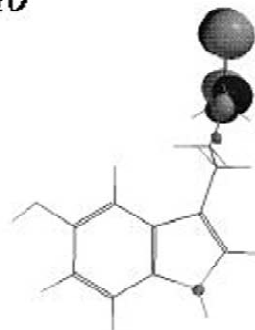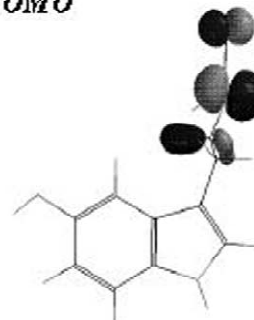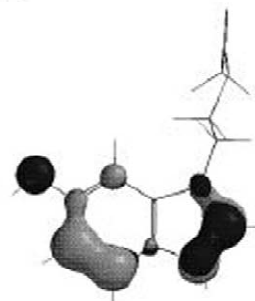
(a)

Fig. 9. Schematic representation of the calculated highest occupied molecular orbitals for the two typical conformers of *N*-acetylserotonin (AS): (a) perpendicular type; (b) parallel type.

of compound AS is 10.6 eV, being close to the ionization energy value relevant to the π_4 (HI) orbital, 10.55 eV. These facts suggest that the 10.1-eV peak of compound M or the 10.6-eV band of compound AS is relevant to the π_4 (MI or HI,

respectively) band. The calculations also give reasonable results: the sixth HOMO of compound M or AS is similar both in shape (Figs. 8 and 9) and in energy to the π_4 (MI or HI, respectively) orbital. Therefore, the remaining lower energy part of the
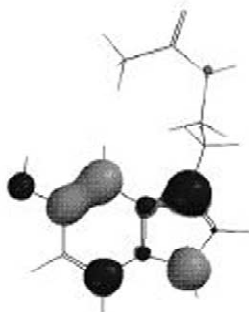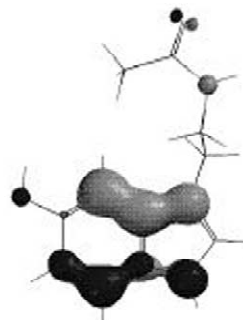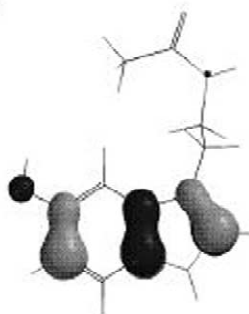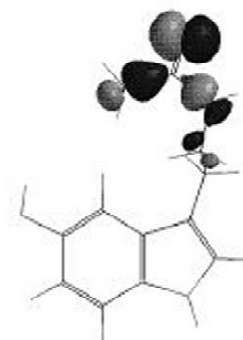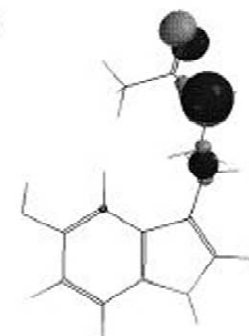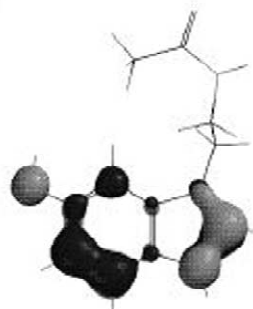

(b)

Fig. 9. (*continued*)

second band group of compound M or the second band group of compound AS is ascribed to the π_3 (MI or HI), π_1 (EA) and $n_{C=O}$ (EA) bands overlapping with one another. As mentioned above, the MO calculations predict some stable molecular conformations of compounds M and AS. The calculations suggest that the orbital interactions among the π_3 (MI or HI), π_1 (EA) and $n_{C=O}$ (EA) orbitals are significant in some cases and negligible in other; these interactions depend strongly on the conformations.

5. Conclusions

The gas phase He(I) photoelectron spectra of melatonin (M) and its related compounds I, HI, MI, EA and AS, have been measured and analyzed with the aid of molecular orbital calculations.

As for the geometrical structure of compounds M or AS, some stable conformations have been predicted by the MO calculations.

The first band group of compounds M or AS has been correlated with the first band groups of compounds MI and HI.

The second band group of compound M and the second and third band groups of compound AS are quite complicated, these bands having been analyzed as follows. The highest energy peak (10.1 eV) in the second band group of compound M or the third band (10.6 eV) of compound AS is relevant to the π_4 (MI or HI, respectively) band. The remaining lower energy part of the second band group of compound M or the second band group of compound AS has been ascribed to the π_3 (MI or HI), π_1 (EA) and $n_{C=O}$ (EA) bands overlapping with one another. According to the calculations, the intramolecular interactions among the π_3 (MI or HI), π_1 (EA) and

$n_{C=O}$ (EA) orbitals are significant in some cases and negligible in others; these interactions depend strongly on the conformations.

References

[1] A.B. Lerner, J.D. Case, Y. Takahashi, T.H. Lee, N. Mori, J. Am. Chem. Soc. 80 (1958) 2587.
[2] P.H. Cannington, N.S. Ham, J. Electron Spectrosc. Relat. Phenom. 32 (1983) 139.
[3] T. Kobayashi, Y. Yokota, S. Nagakura, J. Electron Spectrosc. Relat. Phenom. 2 (1973) 449.
[4] T. Kobayashi, T. Kubota, K. Ezumi, J. Am. Chem. Soc. 105 (1983) 2172.
[5] M. Kubota, T. Kobayashi, J. Electron Spectrosc. Relat. Phenom. 82 (1996) 61.
[6] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, J. Am. Chem. Soc. 107 (1985) 3902.
[7] J.J.P. Stewart, J. Comput. Chem. 10 (1989) 209.
[8] J.J.P. Stewart, J. Comput. Chem. 10 (1989) 221.
[9] SPARTAN, Ver. 4.1, Wavefunction, Inc, Irvine, CA, 1995.
[10] H. Güsten, L. Klasinc, J.V. Knop, N. Trinajstić, Excited states of biological molecules, in: J.B. Briks (Ed.), Proceedings of the International Conference on Excited States of Biological Molecules, Lisbon, 18–24, April 1974, Wiley, Chichester, 1975, p. 45.
[11] H. Güsten, L. Klasinc, B. Ruščić, Z. Naturforsch. 31a (1976) 1051.
[12] J.H.D. Eland, Int. J. Mass Spectometry Ion Physics 2 (1969) 471.
[13] M.H. Palmer, S.M.F. Kennedy, J. Chem. Soc. Perkin Trans. II (1974) 1893.
[14] V.V. Redchenko, T.I. Egorova, V.I. Grigos, B.Ya. Eryshev, Theor. Exp. Chem. 14 (1978) 653.
[15] B. Kovač, L. Klasinc, B. Stanovnik, M. Tišler, J. Heterocycl. Chem. 17 (1980) 689.
[16] L. Klasinc, Pure Appl. Chem. 52 (1980) 1509.
[17] G. Buemi, F. Zuccarello, G. Romeo, J. Mol. Struct. THEOCHEM 14 (1983) 375.
[18] D.A. Sweigart, D.W. Turner, J. Am. Chem. Soc. 94 (1972) 5592.