unknown magnitude of stacking effects as a function of pH.[23] The same difficulty applies to the determination of δ_{II} at zero exchange.

(23) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **87**, 5241 (1965).
(24) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **1**, 1 (1964).
(25) The narrowing of the exchange-broadened resonances into the observable range with the addition of ≥6 M $NaClO_4$ is accounted for quantitatively by the decreased concentration and activity of H_2O, which acts as the major proton acceptor catalyst at low pH (Hoo and McConnell, in preparation). The observed broadening with H_2O as acceptor (eq 1) is in agreement with the broadening calculated from eq 1, where $k_B = k_D \times 10^{(pK_{H_2O} - pK_{NH_2})}$, $k_D = 10^{10}$ for water, $pK_{H_2O} = -1.7$, and $pK_{-NH_2} = 8.55$.[6]
(26) J. J. Mulvihill, *Science*, **176**, 132 (1972).
(27) D. W. Gruenwedel and N. Davidson, *Biopolymers*, **5**, 847 (1967).

Application of Photoelectron Spectroscopy to Biologically Active Molecules and Their Constituent Parts. 6. Opiate Narcotics[1]

L. Klasinc,* B. Ruščić, A. Sabljić, and N. Trinajstić

Contribution from The Rugjer Bošković Institute, P.O.B. 1016, 41001 Zagreb, Croatia, Yugoslavia. *Received December 26, 1978*

Abstract: The He(I) photoelectron (PE) spectra are reported of the free bases of morphine, codeine, heroin, and methadone. They are assigned by the composite molecular method of Rabalais, using the PE spectra of 2-methoxyphenol, 2-methoxy-4-methylphenol, crotyl alcohol, 3-penten-2-ol, allyl acetate, phenyl acetate, diphenylmethane, and 1,1-diphenylacetone to compare the common details of electron structure. The results indicate that their molecular rather than electronic structure is important for analgesic activity.

Opiate narcotics are substances whose actions are similar to those of morphine (Figure 1). Their principal therapeutic use is in the relief of pain.[2] Methadone, although differing considerably from morphine-like narcotics in its chemical structure (Figure 2), exhibits a comparable analgesic potency.[3] It is believed that the valence molecular orbital structure of opiate narcotics plays an important role in the attempt to rationalize their activity.[4] Energies and electron distributions associated with the valence orbitals influence the way in which narcotics participate in weak bonding interactions with their receptors.[5] The electronic structure of opiate narcotics has been extensively investigated in numerous theoretical studies by semiempirical[6-8] and nonempirical[9] molecular orbital calculations. Experimental data are available only for morphine and nalorphine,[9] whose PE spectra have been recorded, although with poor resolution.

Since the calculated results, even those obtained with the nonempirical methods, match poorly the experimental values (~2-eV difference), we decided to compare the PE spectra of the whole series of opiates, starting with the opiate having the simplest structure, and, in order to assign their spectra, to use an empirical approach with qualitative molecular orbital interactions known as the composite molecular approach.[10] The interpretation is simplified by partitioning the complex morphine narcotics into three components: polysubstituted benzene, unsaturated alcohol or ester, and trialkylamine. Only a weak inductive interaction between these components is assumed. The same procedure was used for methadone with the components diphenylmethane, acetone, and trialkylamine.

Experimental Section

Gas-phase He(I) PE spectra were measured with a Vacuum Generators UV-G3 spectrometer.[11] The following compounds were investigated: free bases of morphine, codeine, heroin, and methadone, 2-methoxyphenol, 2-methoxy-4-methylphenol, crotyl alcohol, 3-penten-2-ol, allyl acetate, phenyl acetate, diphenylmethane, and 1,1-diphenylacetone. The PE spectra were measured at room or elevated temperature (morphine, codeine, and heroin at 230 °C, methadone at 120 °C) in the inlet system. Low resolution (30–50 meV) for the complete spectra and high resolution (~15 meV) with an expanded scale for individual systems were used. The energy scale was calibrated using $^2P_{3/2}$ and $^2P_{1/2}$ lines of Xe and Ar. All compounds investigated were of high purity and had been redistilled or recrystallized before use.

Results and Discussion

The spectra of the compounds investigated are shown in Figures 3–6. The vertical ionization energies, defined for the highest peak in a system, are listed above the spectrum. Less certain values are given to one decimal place.

Morphine. The main subunit in the morphine molecule is a polysubstituted benzene. In benzene, the occupied π orbitals, a_{2u} and e_{1g}, give rise in the PE spectrum to systems at 12.35 and 9.23 eV, respectively.[12]

Upon monosubstitution the degeneracy of the e_{1g} benzene orbitals is lifted.[13] In phenol (C_{2v} symmetry assumed), the a_2 component of the e_{1g} benzene orbital does not, for symmetry reasons, interact with the 2p lone pair on oxygen, n_O, and remains at approximately the same energy (9.28 eV) as in benzene. However, the b_1 component is destabilized through antibonding interaction with the n_O orbital (8.56 eV), Figure 7. The remaining two π orbitals in phenol (both of b_1 symmetry) have energies of 11.60 and 13.49 eV.[13b] The first contains mainly an oxygen lone-pair component, while the other corresponds to a stabilized a_{2u} benzene orbital. A similar reasoning attributes to π ionizations the following systems in the PE spectrum of 2-methoxyphenol (Figure 4): 8.13 (π_5), 9.03 (π_4), 11.12 (π_3), 12.5 (π_2), and approximately 14.0 eV (π_1). The top two orbitals can be viewed as antibonding combinations of the components of the benzene e_{1g} orbital with the oxygen lone pairs (Figure 7). Owing to the very low symmetry of the molecule (C_s, at best), the relative ordering of these two orbitals is difficult to infer, but this is not critical. The π_3 and π_2 orbitals are predominantly the negative and positive combinations of the oxygen lone pairs, respectively, while π_1 again corresponds to the a_{2u} benzene orbital. In 2-methoxy-4-methylphenol (Figure 4), introduction of a methyl group at the benzene ring inductively destabilizes the π orbitals relative to 2-methoxyphenol. The destabilizations observed for the top

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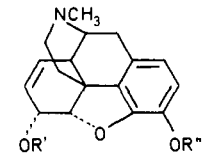


	R'	R"
MORPHINE	H-	H-
CODEINE	H-	CH_3-
HEROIN	CH_3CO-	CH_3CO-

Figure 1. Structure of morphine-like narcotics.

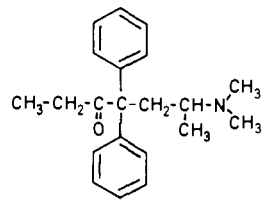


Figure 2. Structure of methadone.

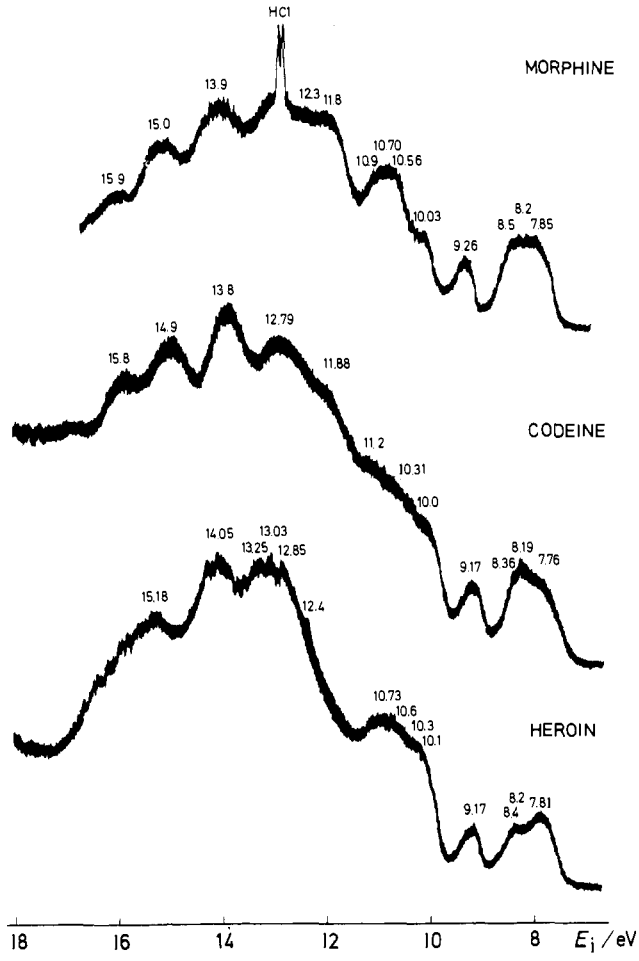


Figure 3. He (I) PE spctra of the free bases of morphine, codeine, and heroin. The HCl in the spectrum of morphine arises from the small amount of hydrochloride in the free base.

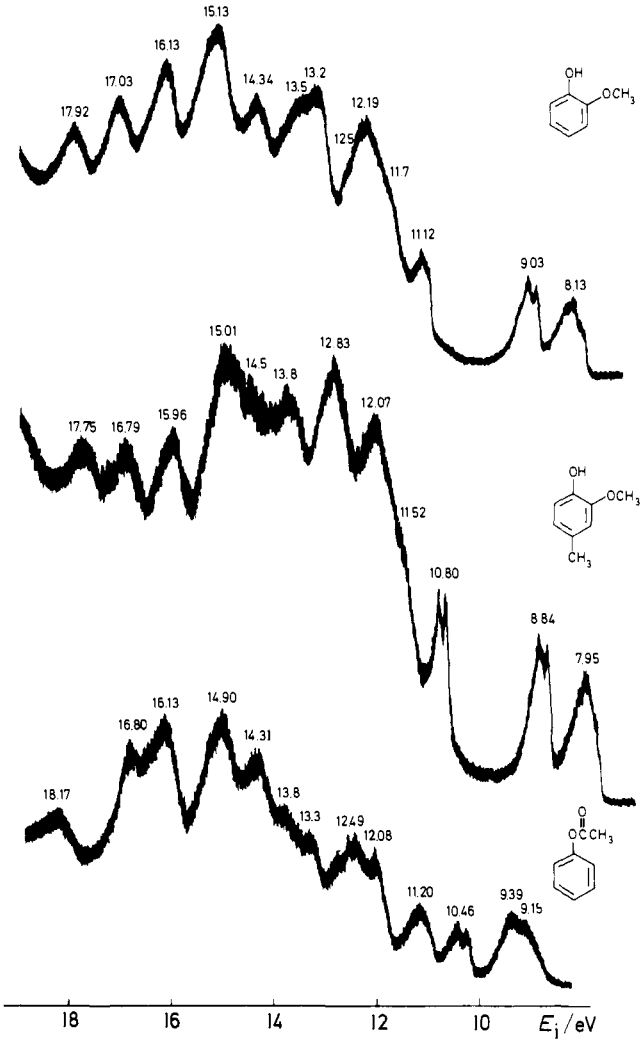


Figure 4. He (I) PE spectra of 2-methoxyphenol, 2-methoxy-4-methylphenol, and phenyl acetate.

three orbitals are 0.18, 0.19, and 0.32 eV, respectively. Assuming further inductive destabilization due to the remaining molecular skeleton of morphine leads to a fair prediction of the energies of the related orbitals in this molecule. Thus, in the PE spectrum of morphine, the systems at 7.85, 8.5, and 10.56 eV can be attributed to π ionizations from the substituted benzene subunit.

The second π subunit in the morphine molecule is an alkyl substituted allyl alcohol. The first system in the PE spectrum of allyl alcohol at 10.16 eV is attributed to ionization from the double-bond orbital ($\pi_{C=C}$), while the relatively sharp system at 10.93 eV is assigned to ionization from n_O.[14] In crotyl alcohol the presence of a methyl group destabilizes these orbitals, and the respective systems in the PE spectrum (Figure 5) are located at 9.70 and 10.70 eV. In 3-penten-2-ol, these orbitals are further destabilized, producing in the PE spectrum (Figure 5) systems located at 9.51 ($\pi_{C=C}$) and 10.52 eV (n_O). Assuming an additional destabilization by the remaining skeleton of the molecule, the systems at 9.26 and 10.03 eV in the PE spectrum of morphine can be attributed to $\pi_{C=C}$ and n_O ionizations, respectively.

In the low-energy region of the PE spectrum of morphine, the only system left so far unassigned is located at 8.2 eV. It can be attributed to ionization from the nitrogen lone-pair orbital (n_N), which favorably compares with the sequence of n_N ionization energies in NH_3 (10.48 eV), NH_2CH_3 (9.64 eV), $NH(CH_3)_2$ (8.97 eV), and $N(CH_3)_3$ (8.44 eV).[15]

An earlier assignment of the PE spectrum of morphine,[9] based on an ab initio calculation with a contracted Gaussian basis set and crystallographic coordinates, disagrees with the present results. Apart from the energy discrepancy between experimental and calculated results, the sequence of the orbitals appears to disagree with experimental evidence even for

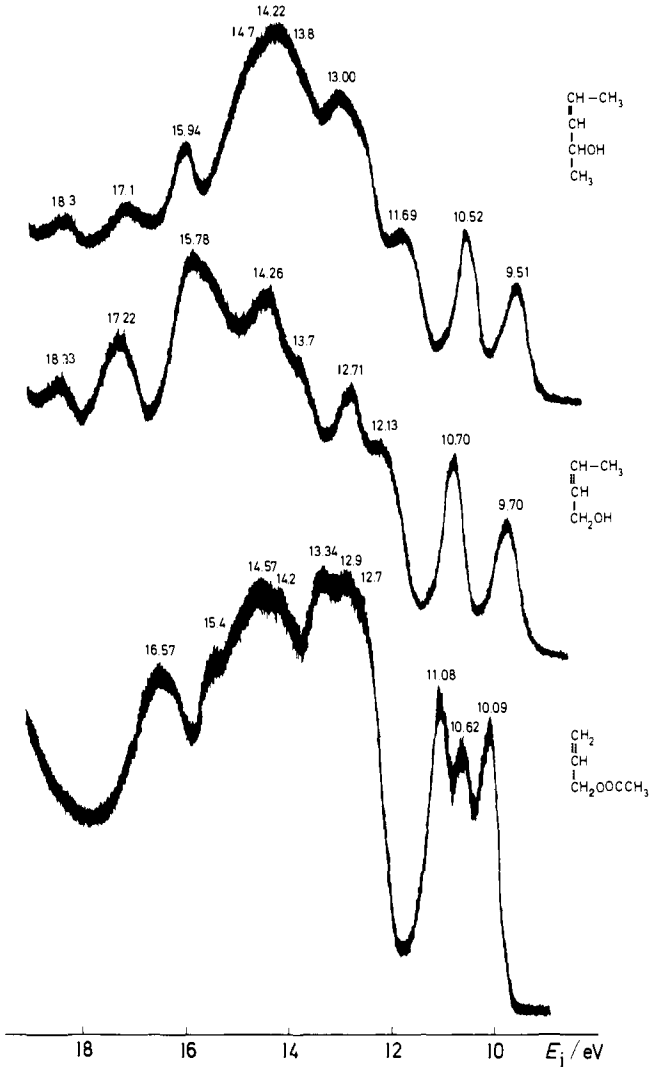


Figure 5. He (I) PE spectra of 3-penten-2-ol, crotyl alcohol, and allyl acetate.

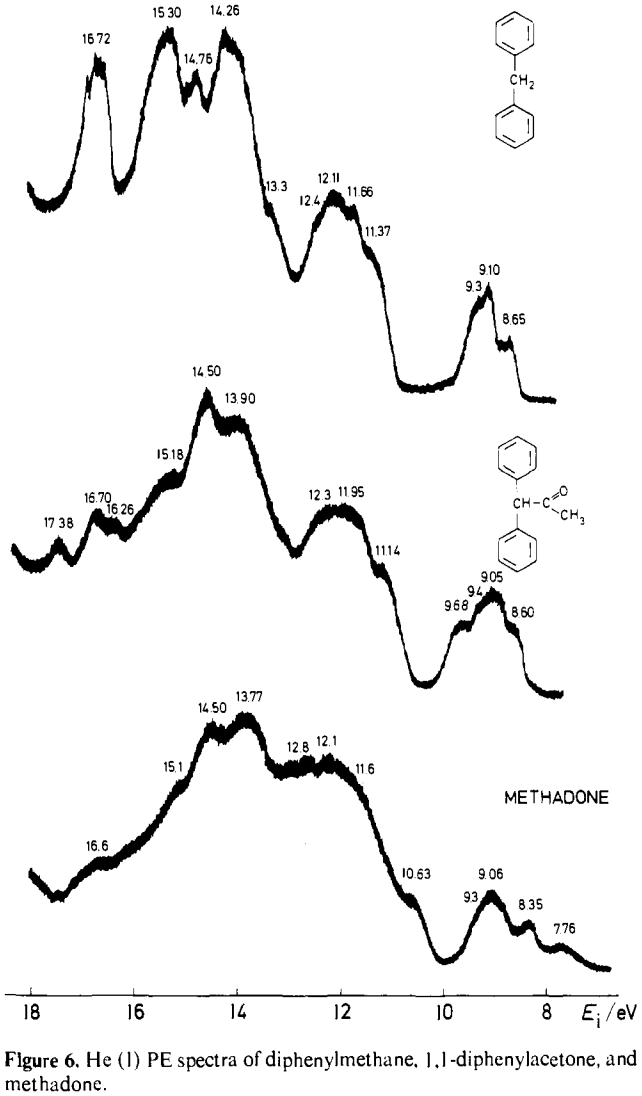


Figure 6. He (I) PE spectra of diphenylmethane, 1,1-diphenylacetone, and methadone.

simple molecules.[15] The allocation of ionization from the second highest occupied molecular orbital (HOMO) at 9.20 eV seems dubious. In the energy range between 7.3 and 8.8 eV two orbitals were assigned, and one between 9.1 and 9.8 eV, while from the corresponding area ratios it follows that three and one, respectively, are observed in the spectrum (Figure 3). Assignment of the system at 9.4 eV to ionization from n_N was based upon its sharpness. Such an assignment disagrees with the experimental result for trimethylamine,[15] where ionization from n_N is very broad (1.5 eV).

Codeine. Codeine can be derived from morphine by methylation of the hydroxylic group in position 3. This methyl group affects mainly the orbitals belonging to the substituted benzene subunit, and destabilizes them by 0.1–0.2 eV (Figure 8). The respective systems in the PE spectrum of codeine (Figure 3) are located at 7.76, 8.36, and 10.31 eV, while the energies of the remaining low-energy systems practically coincide with those of morphine.

Heroin. The major constituent part of the heroin molecule is phenyl acetate. The PE spectrum of phenyl acetate is shown in Figure 4. It can be easily interpreted by comparison with the PE spectra of anisole[13,16] and methyl acetate.[1,17] The top two orbitals of phenyl acetate (9.15 and 9.39 eV) correspond to the benzene e_{1g} orbitals, and are split less than in anisole. The next π orbital (11.20 eV), similar to phenol and anisole, contains mainly an oxygen lone-pair component. The systems at 10.46 and 12.49 eV correspond to ionization from the carbonyl oxygen lone pair ($n_{C=O}$) and carbonyl double bond ($\pi_{C=O}$), respectively. It should be pointed out that similar values for $n_{C=O}$ and $\pi_{C=O}$ ionization were found for carboxylic acid alkyl esters,[1a] thus indicating in the gas phase only a weak π interaction of the carbonyl group with the phenyl ring in the phenyl acetate.

The second major part of the heroin molecule is allyl acetate. Its PE spectrum is presented in Figure 5. In methyl acetate[1,17] the $n_{C=O}$ and n_O ionizations are located at 10.48 and 11.16 eV, respectively, and the $\pi_{C=O}$ ionization is located above 12 eV, while in propylene[18] the $\pi_{C=C}$ ionization is found at 9.86 eV. Thus, it appears that the first system at 10.09 eV in the PE spectrum of allyl acetate corresponds to $\pi_{C=C}$ ionization, the second system at 10.62 eV to $n_{C=O}$ ionization, and the third system at 11.08 eV to n_O ionization. The $\pi_{C=O}$ ionization is located around 12.5 eV, accompanied by σ ionizations. These results indicate, similarly as in phenyl acetate, a negligible π interaction between the carbon–carbon double bond and the carbonyl group in allyl acetate.

Similarly, in heroin, a negligible π interaction between acetyl groups and the remaining part of the molecule can be taken for granted. Thus, its spectrum can be interpreted by comparison with the spectra of morphine and codeine (Figure 3). The new systems in the PE spectrum of heroin, appearing around 10.3 eV, are consequently attributed to $n_{C=O}$ ionizations (Figure 8), while the $\pi_{C=O}$ ionizations are expected at around 12.5 eV.

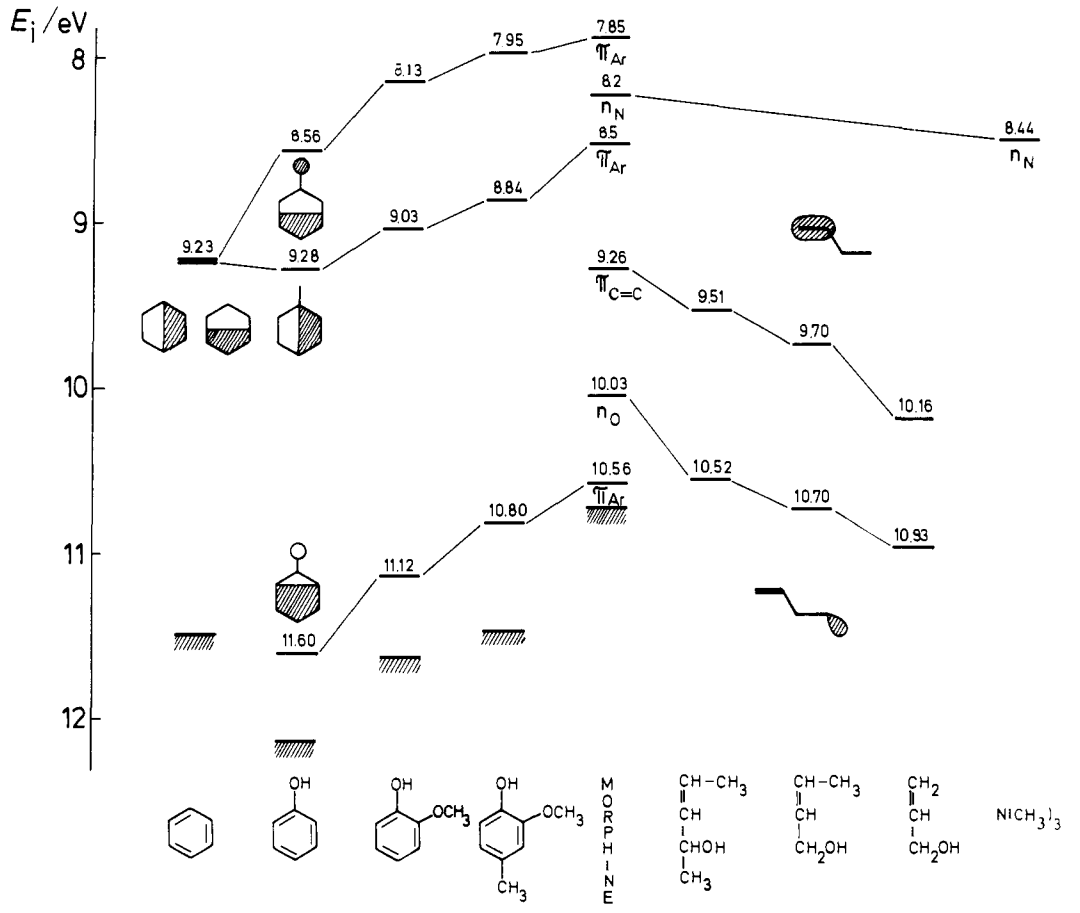


Figure 7. Ionization energy correlation diagram for benzene,[12] phenol,[13] 2-methoxyphenol, 2-methoxy-4-methylphenol, morphine, 3-penten-2-ol, crotyl alcohol, allyl alcohol,[14] and trimethylamine.[15]

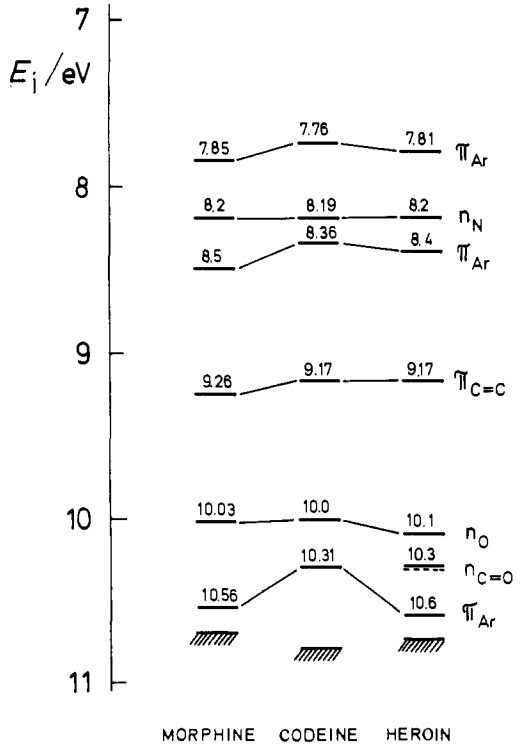


Figure 8. Ionization energy correlation diagram for morphine, codeine, and heroin.

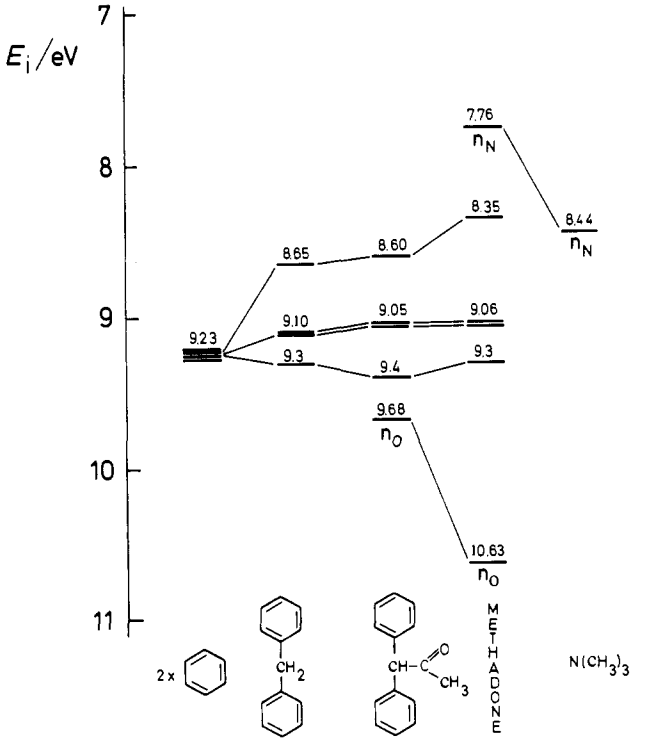


Figure 9. Ionization energy correlation diagram for benzene,[12] diphenylmethane, 1,1-diphenylacetone, methadone, and trimethylamine.[15]

Methadone. In a first approximation, the methadone molecule can be viewed as built from diphenylmethane, dialkyl ketone, and trialkylamine fractions.

In the PE spectrum of diphenylmethane (Figure 6) the six systems at 8.65, 9.10 (two unresolved), 9.3, 11.37, and 13.3 eV, can be attributed to π ionizations. They are assigned in the

Table I. Activity of Analgesics in Mice and in Men[28]

substance	mice ED_{50}, mg/kg	Men equivalence to 10 mg of morphine, mg	ref
morphine	2.1	10	3, 32
codeine	14.2	60-120	3, 32
heroin	0.9	3-5	3, 32
methadone	1.6	10	3, 33

same manner as biphenyl and fluorene[20] were taking diphenylmethane as two weakly interacting benzene subunits (Figure 9). The systems at 8.65, 9.10, and 9.3 eV arise from the antibonding, the two nonbonding, and bonding combinations of appropriate components of interacting benzene e_{1g} orbitals, respectively. Similarly, the antibonding and bonding combinations of two benzene a_{2u} orbitals are responsible for the systems at 11.37 and 13.3 eV, respectively. The observed shift of the centers of gravity of these systems relative to benzene (Figure 9) is the result of inductive destabilization by the methylene link. The interaction of an α-carbonyl group with the phenyl rings can be seen from the PE spectrum of 1,1-diphenylacetone (Figure 6). Here, the systems corresponding to phenyl π orbitals are found at nearly the same energies as in diphenylmethane. The new system at 9.68 eV is attributed to $n_{C=O}$ ionization. This energy is the same as in acetone (9.66 eV[17,19]), thus confirming that there is no significant through-space interaction of the carbonyl group with the phenyl rings.

The comparison of the low-energy region of the PE spectra of methadone (Figure 6) and 1,1-diphenylacetone shows that the energies of the π orbitals nearly coincide, except for the highest one, which is destabilized by 0.25 eV in the former compound. The very broad top system centered at 7.76 eV in the PE spectrum of methadone corresponds mainly to n_N ionization, while the $n_{C=O}$ ionization appears at 10.63 eV. These values differ from those in trimethylamine (8.44 eV) and 1,1-diphenylacetone (9.68 eV). This fact may represent a strong through-space interaction of the n_N and $n_{C=O}$ lone pairs, which is possible if they are sufficiently close to each other in the gas phase. This tentative explanation is supported to some extent by the X-ray crystallographic study of methadone base,[21] which indicates the formation of a five-membered ring by nitrogen-carbonyl carbon interaction. Perhaps the methadone base prefers a similar conformation in the gas phase, too.

Structure-Activity Considerations. During the past 2 decades a number of correlations between calculated HOMO energies and activity of psychotropic drugs have been reported in the literature.[22-25] Houk and co-workers[26] proposed recently a model in which they have correlated the average of two highest occupied MOs with activity of psychotropic drugs. This model works for phenylamines, tryptamines, and LSD,[26] but fails in the case of phenothiazine and related tranquilizers.[27]

The activity of opiate narcotics is presented in Table I.[28] Virtually none of the proposed models works in this case, probably for two reasons: the number of investigated opiates is rather small, and the differences between the E_i's of very broad bands are subtle (0.1 eV). Thus, it could be said that all have essentially identical E_i's and electronic structures and this represents an important situation: any difference in activity must be due to direct interactions of the substituent with the receptor, or direct effect on some physical property. The activity measures are both whole animal activities, and perhaps the binding affinities would be more similar than the whole animal activities. However, qualitative differences in activity between examined narcotics due to direct interactions of the substituents with the receptor can be rationalized with the help of an opiate receptor model (lock and key model).[29] According to this model the receptor achieves selectivity through (at least) three regions of interaction. Taking morphine as the prototype for the opiate narcotics, these regions would be substituted benzene, amino group, and unsaturated alcohol. Codeine possesses an order of magnitude lower activity than morphine (Table I). The slight destabilization of the HOMOs (methyl effect) cannot explain such a drastic decrease in activity. A tentative explanation is, perhaps, connected with the steric hindrance of the benzene moiety by the methyl group, which weakens the interaction with the receptor. Consequently, the heroin molecule, with its two additional carbonyl groups, is able to saturate a greater number of sites than the morphine molecule and adequately exhibits higher activities. Although differing considerably in the chemical structure, there is good evidence that methadone is acting at the same receptor sites as the opiates.[30] This is achieved through mimetization of the morphine structure (zipper model),[8,31] the three interacting regions being the phenyl ring, amino group, and carbonyl group, and it is not surprising that it exhibits a comparable analgesic activity. Thus, the possibility that the molecular rather than the electronic structure is more important for the analgesic activity of the studied opiates should also be considered and is indicated by the results presented here. However, this does not exclude the possibility that other aspects of the biochemistry of these opiates, for example, metabolic steps leading to elimination, may indeed be influenced electronically.

References and Notes

(1) (a) Part 5: L. Klasinc, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **5**, 373 (1978). (b) Reported in part at the 8th and 9th Yugoslav Symposia on Biophysics, 1977 and 1978.
(2) A. K. Reynolds and L. O. Randal, "Morphine and Allied Drugs", University of Toronto Press, Toronto, 1957.
(3) N. B. Eddy, H. Halbach, and O. J. Braenden, *Bull. W. H. O.*, 353 (1956).
(4) J. J. Kaufman, E. Kerman, and W. S. Koski, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **1**, 289 (1974).
(5) G. H. Loew, D. Berkowitz, H. Weinstein, and S. Srebrenik in "Molecular and Quantum Pharmacology", E. D. Bergmann and B. Pullman, Eds., Proceedings of the 7th Jerusalem Symposium on Quantum Chemistry and Biochemistry, D. Reidel Publishing Co., Dordrecht, Holland, 1974, p 355.
(6) J. J. Kaufman and W. S. Koski, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **2**, 35 (1975).
(7) G. H. Loew and D. S. Berkowitz, *J. Med. Chem.*, **18**, 656 (1975).
(8) G. H. Loew, D. S. Berkowitz, and R. C. Newth, *J. Med. Chem.*, **19**, 863 (1976).
(9) H. E. Popkie, W. S. Koski, and J. J. Kaufman, *J. Am. Chem. Soc.*, **98**, 1342 (1976).
(10) J. W. Rabalais, "Principles of Ultraviolet Photoelectron Spectroscopy", Wiley, New York, 1977, p 286.
(11) L. Klasinc, B. Kovač, and B. Ruščić, *Kem. Ind.*, **23**, 569 (1974).
(12) D. M. W. Van der Ham, M. Beerlage, D. Van der Meer, and D. Feil, *J. Electron Spectrosc. Relat. Phenom.*, **7**, 33 (1975).
(13) (a) J. P. Maier and D. W. Turner, *J. Chem. Soc., Faraday Trans. 2*, **69**, 521 (1973); (b) L. Klasinc, unpublished results.
(14) A. Katrib and J. W. Rabalais, *J. Phys. Chem.*, **77**, 2358 (1973).
(15) K. Kimura and K. Osafune, *Mol. Phys.*, **29**, 1073 (1975).
(16) P. Kobayashi and S. Nagakura, *Bull. Chem. Soc. Jpn.*, **47**, 2563 (1974).
(17) J. L. Meeks, H. J. Maria, P. Brint, and S. P. McGlynn, *Chem. Rev.*, **75**, 603 (1975).
(18) R. A. Wielesek and T. Koenig, *Tetrahedron Lett.*, 2429 (1974).
(19) The PE spectrum of benzyl methyl ketone has been recorded, too, and it shows only weak interaction of the phenyl ring with the carbonyl group in the α position.
(20) B. Ruščić, B. Kovač, L. Klasinc, and H. Güsten, *Z. Naturforsch. A*, **33**, 1006 (1978).
(21) H. B. Burgi, J. D. Dunitz, and E. Shefter, *Nature (London), New Biol.*, **244**, 187 (1973).
(22) G. Karreman, I. Isenberg, and A. Szent-Györgi, *Science*, **130**, 1191 (1959).
(23) S. H. Snyder and R. C. Merril, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 285 (1965).
(24) S. Kang and J. P. Green, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 62 (1970).
(25) S. Kang and J. P. Green, *Nature (London)*, **226**, 645 (1970).
(26) L. N. Domelsmith, L. L. Munchausen, and K. N. Houk, *J. Am. Chem. Soc.*, **99**, 4311 (1977).
(27) L. N. Domelsmith, L. L. Munchausen, and K. N. Houk, *J. Am. Chem. Soc.*, **99**, 6506 (1977).
(28) A. E. Jacobson, E. L. May, and L. J. Sargent, "Medicinal Chemistry", Vol.

II, Wiley, New York, 1970, pp 1927–1950.
(29) A. F. Feinberg, I. C. Creese, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 4215 (1976).
(30) H. W. Kosterlitz and A. A. Waterfield, *Annu. Rev. Pharmacol.*, 29 (1975).
(31) A. S. V. Burger, G. C. K. Roberts, and J. Feeney, *Nature (London)*, **253**, 753 (1975).
(32) E. L. May and L. J. Sargent, in "Analgesics", Vol. 5, G. de Stevens, Ed., Academic Press, New York, 1965, Chapter IV.
(33) R. A. Hardy, Jr., and M. G. Howell in ref 32, Chapter V.

Fluorine Nuclear Relaxation Studies of *p*-Trifluoromethylbenzenesulfonyl-α-chymotrypsin

J. T. Gerig,* D. T. Loehr, K. F. S. Luk, and D. C. Roe

Contribution from the Department of Chemistry, University of California, Santa Barbara, Santa Barbara, California 93106. *Received May 7, 1979*

Abstract: Spin-lattice and transverse fluorine relaxation rates have been determined for the title enzyme derivative at pH 7. The data have been analyzed to provide an estimate of the rotational correlation time (τ_c) near the trifluoromethylbenzenesulfonyl group and the correlation time (τ_i) for internal rotation of trifluoromethyl. The major part of the fluorine relaxation is due to proton-fluorine dipole-dipole interactions. Specific deuteration experiments show that these interactions predominantly involve protons of the enzyme and solvent.

The use of spectroscopic reporter groups is an important aspect of modern protein biophysical chemistry and the past decade has seen an increasing utilization of reporter groups which are amenable to study by magnetic resonance techniques. These latter experiments are especially attractive because they often provide information about the dynamics of molecular motion near the reporter group and, by inference, of the protein itself.

Fluorine-substituted moieties can be introduced readily into protein structures, thereby providing materials which can be examined by fluorine magnetic resonance (^{19}F NMR) spectroscopy.[1] ^{19}F NMR in these systems offers the advantage of ease of signal detection as well as being characterized by chemical-shift effects and relaxation rates that are highly sensitive to the environment of the reporter (fluorine) nucleus.

Although covalently bound fluorine is similar in steric bulk to covalent hydrogen, fluorine is highly electronegative and potentially a hydrogen-bond acceptor. Fluorine substitution within the confines of a tightly structured protein may exert an effect on the structure of the protein, rendering data obtained by ^{19}F NMR spectroscopy irrelevant to the properties of the unmodified system. It is, therefore, necessary to have information on the possible structural consequences of fluorine substitution in a variety of biomolecular systems so that these perturbations may be recognized and possibly avoided.

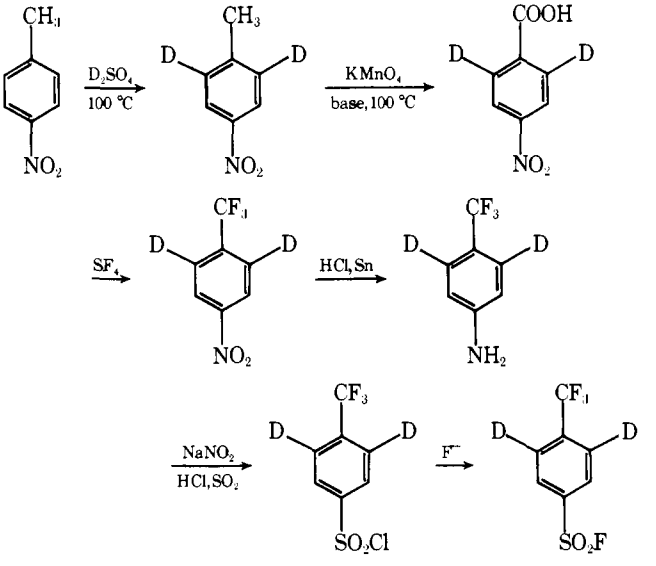
The structure of the enzyme α-chymotrypsin has been investigated by a large number of experiments, including X-ray crystallography, and this work has provided a foundation for a detailed understanding of the mechanism of action of the protein.[2] An X-ray structure of tosylchymotrypsin, a derivative in which the serine-195 residue at the active site has been esterified, is also available.[3] We have previously reported the preparation and purification of α-chymotrypsin which had been inactivated by treatment with *p*-trifluoromethylbenzenesulfonyl fluoride.[4,5] This protein can be regarded as an analogue of tosylchymotrypsin in which a methyl group has been replaced by trifluoromethyl. In this and subsequent papers we report ^{19}F NMR studies of this protein in solution; the results bear on the question of the effects of fluorine substitution on protein structure and provide information about the dynamics of molecular motions at the protein active site.

Experimental Section

Materials. 4-Trifluoromethylbenzenesulfonyl fluoride was prepared as described previously.[4]

3,5-Dideuterio-4-trifluoromethylbenzenesulfonyl fluoride was synthesized according to the reactions in Scheme I. Dideuterated 4-nitrotoluene was obtained by heating 21 g (0.18 mol) of 4-nitrotoluene (Aldrich) in 70 g of deuterium sulfate (Stohler, 99% D) according to the procedure of Renaud et al.[6] After three exchanges, the ^{1}H NMR spectrum of the product showed a single resonance in the aromatic region of the spectrum at the chemical shift of the 2,6 protons. 3,5-Dideuterio-4-nitrobenzoic acid was obtained from this material by oxidation of 15 g (0.1 mol) with 40 g of potassium permanganate using the procedure of Bigelow.[7] The acid (9 g, 0.05 mol) was treated with sulfur tetrafluoride (Matheson, 14 g, 0.13 mol) in a stainless steel vessel at 135 °C for 20 h.[8] After this time, excess SF_4 was removed and the residual oil taken up in 100 mL of ether. The ether layer was dried over magnesium sulfate and the solvent removed in vacuo, leaving a crude product (mp 35–40 °C, lit. 41–43 °C[9]) which was used directly in the reduction step. 2,6-Dideuterio-4-aminobenzotrifluoride was obtained by reduction of crude 4-nitrobenzotriflu-

Scheme I. Preparation of 3,5-Dideuterio-4-trifluoromethylbenzenesulfonyl Fluoride



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