*3,6-Di-O-methyl-D-mannose* was prepared from VIII by acid hydrolysis as described above. The substance, which did not crystallise, showed $[\alpha]_D+4°$ (*c*, 0.8, water) in agreement with a previously determined value.[4]

*GLC-MS.* The 2,4- and 3,6-di-*O*-methyl-D-mannoses were converted into the corresponding alditol acetates, which were chromatographically homogeneous on GLC. The retention times, on an ECNSS-M-column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol,[5] were 5.44 and 4.15, respectively. The fragmentation patterns on MS were indistinguishable from those of other, authentic 2,4- and 3,6-di-*O*-methyl hexitol acetates,[8] respectively.

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# The Valence Electron Density Distribution of Strained Single Bonds in the Iterative Extended Hückel Approach

## IV. A Comment on the Choice of Slater Exponents in the Calculations of Electron Densities

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Slater orbitals are often used in molecular calculations. In these cases orbital exponents obtained by the so-called Slater's rules (see for instance Ref. 1) are generally applied, although these values were originally created for atomic purposes. For hydrogen the difference between the so obtained "atomic" and suggested "molecular" values is not negligible, Slater's rules giving 1.0 as compared to 1.2 obtained by minimizing the energy of the hydrogen molecule.[2] As regards the electron density distribution, a greater value of the exponent of the hydrogen orbitals will concentrate the charge somewhat more at the hydrogen atoms.

In order to ensure that the principal results obtained earlier by using the value 1.0[3] for the hydrogen Slater exponent are not violated by a change to 1.2, some recalculations have been made applying the suggested value 1.2 in the calculations of the eigenvectors as well as in the evaluation of the density function. From Figs. 1, 2, and 3 we see that the general picture of the valence electron density distribution of methane in its normal state and under deformation (at angles of 120° and 60°, respectively, mutually between the symmetrically moved three interatomic vectors, *i.e.* 90° and 140°, respectively, between the moving vectors and the fixed one) is essentially the same as in the preceding calculations, the difference being merely a higher charge at the sites of the hydrogen atoms. (It should be pointed out that the levels are not exactly the same in the two

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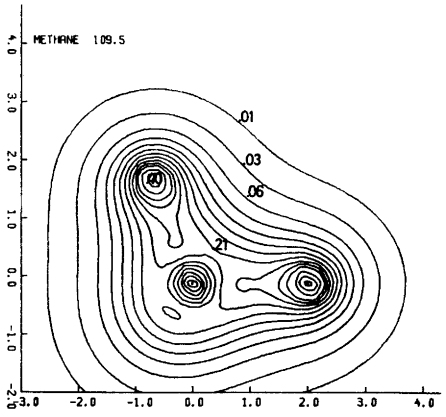


*Fig. 1.* Valence electron density of methane. No deformation.

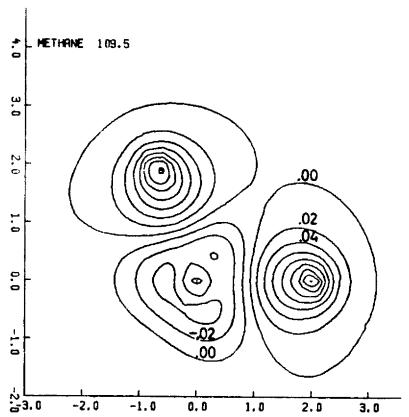


*Fig. 4.* Valence electron density difference of methane. No deformation.

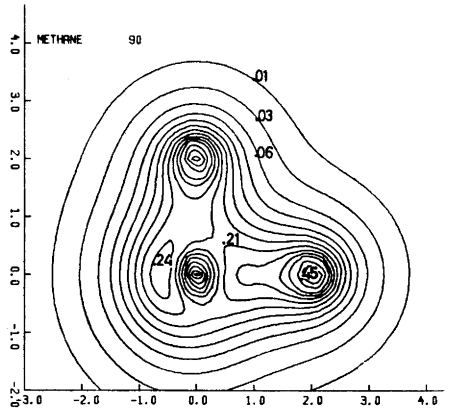


*Fig. 2.* Valence electron density of deformed methane. Valence angle 90°.

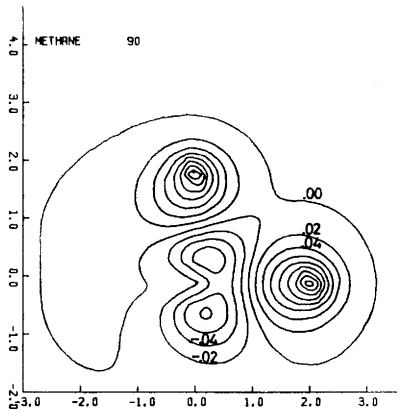


*Fig. 5.* Valence electron density difference of deformed methane. Valence angle 90°.

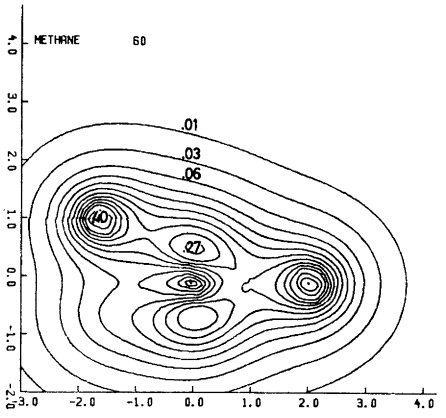


*Fig. 3.* Valence electron density of deformed methane. Valence angle 140°.

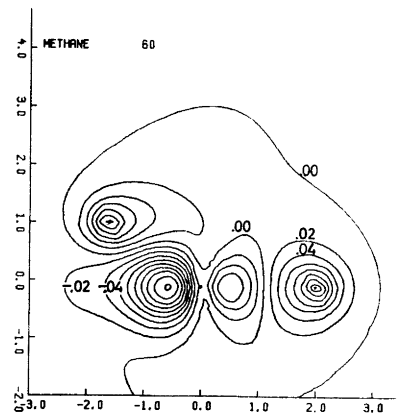


*Fig. 6.* Valence electron density difference of deformed methane. Valence angle 140°.

versions being in this paper 0.1, 0.3, 0.6,...0.30, 0.35, 0.40, 0.45.)

In a recent work, Bader and Preston report their results from the determination of the charge density distribution in methane, not by energy minimization but by the requirement that the charge reproduces observed values of properties which are themselves functions of the one-electron charge density.[4] They obtained a charge density of 0.40 a.u. at the centers of the hydrogen atoms which is in good agreement with those reported here.

The corresponding density differences have also been recalculated whereby the molecular density is based on the "molecular" exponent 1.2 but the atomic densities to be subtracted on the "atomic" value 1.0 for the hydrogen atoms. Figs. 4, 5, and 6 show that the density difference, as expected, has maxima which now are centered on or near the sites of the hydrogen atoms.

As far as the general picture of strain is concerned, the choice of exponent for the hydrogen 1*s* orbital in the calculation of the valence electron charge density does not seem to be essential.

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# Activation of Chymotrypsin by Formation of a Covalent Dimer

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The activity of Chymotrypsin (CT) depends upon the existence of an ionic linkage between ile-16-$NH_3^+$ and asp-194-$COO^-$ which stabilizes the active site.[1,2] The native enzyme exists as a mixture of two isomers, one of which possesses the ionic linkage and is active whereas the other lacks the ionic linkage and is inactive.[3] Binding of a specific substrate or inhibitor displaces the equilibrium towards the active form. This process, which is accompanied by rotation of the asp-194 side-chain and relief of self-inhibition was detected by optical rotatory dispersion,[4] X-ray diffraction,[3] *etc.*[1,3,5] The purpose of this work was to establish the role of the ile-16 amino group in the catalytic process by anchoring it in its charged form as an amidine. Such a modification might eliminate the conformational change and influence the activity. Removal of the charge on the amino group by acylation inactivated the enzyme whereas monofunctional amidination did not affect the activity.[5] Selective, bifunctional amidination of ile-16-$NH_2$ of $\delta$-CT by malonic di-imidoester produced an inactive monomer and a dimer with strongly enhanced esterase (500—1000 fold) and reduced amidase (*ca.* 10 % left) activity. Similar, but less pronounced effects were obtained with succinic di-imidoester. The modification was performed as follows:

The amino groups of twelve times recrystallized chymotrypsinogen were exhaustively acylated with dimethylmaleic anhydride at pH 8.8—9.2 at 4°C for 10 min.[6] Excess reagent and byproducts were removed by dialysis. The zymogen was then activated by trypsin to a $\delta$-CT which only possesses one amino group (ile 16).[7] This was amidinated by malonic di-imido ester [8] and dialyzed at pH 3 to remove byproducts and deacylate the amino groups.[13] Finally, the preparation (MCT) was fractionated on a calibrated Sephadex column. About 85 % was active dimer whereas the remainder was inactive monomer. As the amidination reagent might be sufficiently nucleophilic to displace the acyl blocks,