was obtained also from the submerged water plant *Vallisneria spiralis* (ARISZ and SOL[39]). Accumulation of chloride in the cytoplasm as well as in the vacuole was also reported for *Nitella translucens* (MACROBBIE[40–42]). High chloride concentrations were also postulated by MACROBBIE[40] for the chloroplasts of *Nitella translucens*. Other reports claimed that in cells of halophytes the cytoplasm was rich in chloride and that chloride was maintained there as free ions (ARNOLD[2]; OSMOND et al.[5]).

Chloride distribution in *Potamogeton lucens* did not agree completely with either of the previously reported assumptions. In leaf-cells of *Potamogeton* the highest signals for chloride always appeared either in the vacuole or the cell wall (free space) fraction. Only very low signals of chloride were detected in the chloroplasts, and the connection between chloride uptake and electron transport in those chloroplasts would thus be questionable.

In summary, it is evident that individual cells of *Potamogeton* vary in their content as well as in their ion distribution, Thus, an investigation into the localization of ions inside subcellular organelles of plants must consider difficulties arizing from technical limitations, as well as from the high variability among cells, and tissues.

Zusammenfassung. Die Verteilung von Natrium, Kalium, Kalzium, Magnesium und Chlor wurde in Mesophyllzellen der Blätter von *Potamogeton lucens* mit Hilfe der Röntgen-Mikrosonde untersucht. Die Blattzellen dieses Wasserpflanze variierten beträchtlich in bezug auf Menge und Verteilung der Ionen. In den meisten Zellen wurden im Cytoplasma und in den Chloroplasten hohe Werte für Natrium und niedrige für Chlor beobachtet. In den Vakuolen wurden für sämtliche untersuchten Ionen hohe Werte gefunden.

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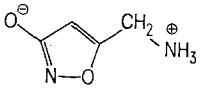
COGITATIONES

Molecular Orbital Studies on the Conformations of Bicuculline and β-Hydroxy GABA[1]

In a previous study we reported molecular orbital calculations of the preferred conformation of γ-aminobutyric acid (GABA) and muscimol (I)[2]. That study predicted an extended chain for the GABA zwitterion with free rotation for the carboxylate group. The distance separating the onium group from one carboxylate oxygen ranged from 5 to 6 Å. This was postulated to be a critical pattern or pharmacophore for GABA activity. In the same study the GABA agonist muscimol (I) was predicted to prefer a conformation in which the side chain is coplaner with the ring and bent toward the ring oxygen atom. The distance separating the exocyclic ring and onium group was predicted to be 5.8 Å, in good agreement with our postulated GABA pharmacophore[2].



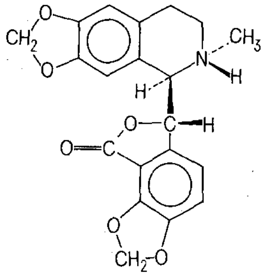
(I)

A recent study of the crystal conformation of GABA has been reported using X-ray analysis[3]. This study reports a folded molecule with the onium group-oxygen separation 4.5 Å or less.

An X-ray analysis of the muscimol crystal reveals that the side chain is bent toward the ring oxygen atom but is 60° out of the ring plane[4]. The exocyclic oxygen to onium group distance was found to be 5.77 Å, in excellent agreement with our prediction.

Experimental support for our proposed GABA pharmacophore comes from evidence that 4-amino -2-butynoic acid is a GABA agonist[5]. Assuming that this rigid molecule exists in solution as a zwitterion, the separation of the onium group from a carboxylate oxygen atom is fixed between 5.2 and 5.8 Å, in good agreement with our postulated GABA pharmacophore.

In an effort to further examine the validity of our GABA hypothess we have periformed calculations on the GABA agonist β-hydroxy GABA[6] and the GABA competitive antogonist, bicuculline (II)[7]. A possible receptor equivalence has been speculated upon in which the carbonyl oxygen to onium group distance in bicuculline may be comparable to the corresponding structural features in GABA[7].



(II)

The calculations were performed using extended Huckel theory[8] with parameters previously employed[9]. The dimensions used were the standard bond lengths and angles proposed for M.O. calculations and used extensively by us[10]. Angular increments of 30° were used.

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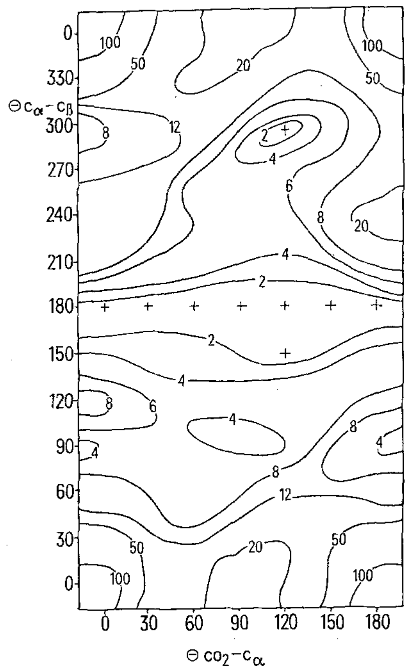

Fig. 1. Calculated energy vs. angle contour map for β-hydroxy GABA. The figure shows the relationships for the carboxylate rotation, θ co$_2$-c$_\alpha$, and the α and β carbon relationship, θ c$_\alpha$–c$_\beta$.

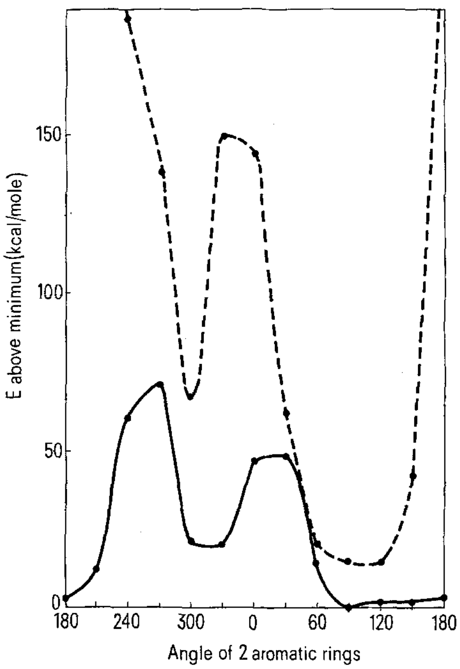

Fig. 2. Calculated energy vs. angle plot for the ring juncture bond in bicuculline. The dashed line is the energy for the proton-lactone *trans*-isomer. The solid line is the energy for the proton-lactone *cis*-isomer.

The results of the calculations on β-hydroxy GABA revealed two equivalent conformational preferences, Figure 1. The first conformation finds the molecule in an all trans conformation (θ c$_\alpha$–c$_\beta$ = 180°) and the carboxylate group free to rotate (θ co$_2$ – c$_\alpha$ = 0° – 180°). The second conformation is that in which the C$_\alpha$–C$_\beta$ bond is gauche (θ c$_\alpha$ – c$_\beta$ = 300°) with the hydroxyl group close to the carboxylate group. The carboxylate group is inclined toward the hydroxyl group in a single conformation (θ Co$_2$ – C$_\alpha$ = 120°). This hydroxyl-carboxylate proximity way well be due to hydrogen bonding. This second preference resembles the folded conformation found for the GABA crystal[3]. In the trans conformation the onium to carboxylate oxygen distance is identical to that found for GABA, 5–6 Å depending upon the carboxylate oxygen considered[11].

The calculations on bicuculline were made on the protonated molecule. The configuration used was that derived by NMR analysis, Figure 2. There remains the ambiguity of the position of the proton on the nitrogen atom. It was necessary to perform calculations in which the proton was *cis* and *trans* to the lactone ring. The calculated preferred conformation occurs when the proton is *cis* to the lactone ring and with the dihedral angle between the aromatic rings 90°, as shown in (II) and Figure 2. In this conformation, the onium group to carbonyl oxygen atom is about 5.6 Å. If we assume that these features are the receptor equivalents of the onium group and carboxylate oxygen in GABA, then it is apparent, that bicuculline possesses structural features and dimensions which could impart receptor activity to it as a competitive antagonist.

The results of these calculations support our GABA pharmacophore hypothesis[2].

Zusammenfassung. Mit Hilfe der MO-Theorie wurden bevorzugte Konformationen für den GABA Agonisten β-Hydroxy-GABA und den Antagonisten Bicucullin berechnet. In beiden Fällen ergeben sich fast gleiche Abstände zwischen der Ammoniumgruppe und dem Sauerstoffatom wie in der entsprechenden Struktur von GABA selbst.

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PRO EXPERIMENTIS

Preparative Block Electrophoresis Using Sephadex G-25

Considering the widespread application of Sephadex for preparative gel-filtration, it is somehow surprising that so few attempts to use this material as support medium for preparative electrophoresis have been reported[1-6]. For this purpose, Sephadex G-25 appears to be the ideal grade, since it could be expected that most proteins would be excluded from the particles, separation being primarily determined by charge alone, and recovery of