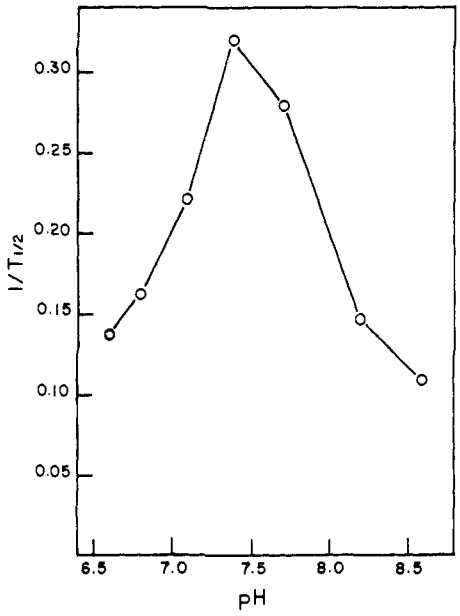

Figure 5. pH vs. rate of inactivation; 0.05 units of enzyme was incubated with 5×10^{-7} *M* gabaculine in 0.1 *M* potassium phosphate buffer at pH's 6.6, 6.8, 7.1, 7.4, and 7.8 and potassium pyrophosphate buffer at pH's 8.2 and 8.6 at 37 °C. The rates of inactivation of the enzyme were measured and the $t_{1/2}$'s determined. $1/t_{1/2}$ is plotted vs. pH above. The same curve is generated at saturating levels of gabaculine.

The initial step in the inactivation process leads to the rate limiting formation of the enzyme bound, transaminated product **3.** Sequence A would involve a Michael addition of an active-site nucleophile to this activated intermediate to generate the irreversibility inhibited enzyme **4.** This mode of inhibition has precedent in the irreversible inactivation of pyridoxal linked aspartate aminotransferase by β,γ-unsaturated amino acids.[7] Sequence B would involve the simple aromatization of **3** to yield the *m*-anthranilic acid derivative **5.** This process would be expected to occur spontaneously if a Lewis base happens to be adjacent to the 2-C–H bond of **3** in the enzyme-inhibitor complex. This process would also yield an irreversibly inactivated enzyme because this complex would remain enzyme bound. Pyridoxal phosphate itself strongly resists resolution from the native holoenzyme. Further experiments are in progress to decide which of these two mechanisms, if either, best accomodate the data.

Both mechanisms require the direct irreversible inhibition of the enzyme prior to the dissociation of the enzyme inhibitor complex **3.** We can rule out other mechanisms that would produce a diffusible inhibitor in two ways. First of all, exogenous trapping agents such as mercaptoethanol have no effect on the rate of inactivation. Secondly, successive additions of enzyme to an excess of inhibitor all show the same rates of inhibition.[6] A mechanism that postulates a reactive intermediate in solution would predict that each succeeding addition of enzyme would be inhibited more rapidly than the previous one.

Gabaculine is likely to be useful in defining the physiological roles of the inhibitory neurotransmitter GABA in vivo. We have found that gabaculine irreversibly inhibits mouse brain enzyme in vivo. It should be noted that the most specific irreversible inactivator of GABA transaminase to date, 4-amino-5-hexynoic acid, a very cleverly designed inhibitor which also functions by a mechanism requiring catalytic turnover, is two to three orders of magnitude less potent than is gabaculine in vitro.[4]

In conclusion, we have demonstrated that the natural product gabaculine is a potent, specific irreversible inhibitor of mammalian GABA transaminase both in vitro and in vivo. This compound is a further example of a naturally occurring irreversible inhibitor that functions by a novel mechanism requiring the enzyme to catalyze its own destruction.

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Additions and Corrections

Stereochemistry of Metal Anion Substitutions on Carbon [*J. Am. Chem. Soc.*, **94**, 7205 (1972)]. By G. S. KOERMER, M. L. HALL, and T. G. TRAYLOR,* Department of Chemistry, Revelle College, University of California, San Diego, La Jolla, California 92093.

The last entry in Table I is incorrect. No 4-camphyltrimethyltin was obtained. We have since found that, whereas bridgehead bromides react readily with Me_3SnLi, bridgehead chlorides are unreactive.

Photochemical Rearrangement of *β*-*tert*-Butylstyrenes. Stereochemistry [*J. Am. Chem. Soc.*, **95**, 3031 (1973)]. By STEPHEN S. HIXSON* and TIMOTHY P. CUTLER, Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01002.

Substituent Effects on π–π^* Rearrangements. Methyl Migration in *β*-*tert*-Butylstyrenes [*J. Am. Chem. Soc.*, **95**, 3032 (1973)]. By STEPHEN S. HIXSON* and TIMOTHY P. CUTLER, Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01002.