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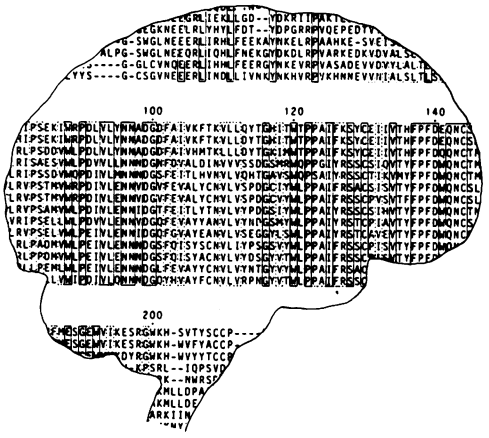

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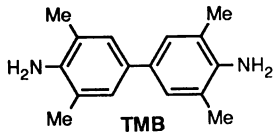
Tetramethylbenzidine

A reported noncarcinogenic analog of benzidine



TMB

For many years benzidine was used as a sensitive and specific reagent for the detection of blood.[1] However, in 1974 the Occupational Safety and Health Administration banned its manufacture and use in the United States because of its extreme carcinogenicity.[2]

One of the early hypotheses for the carcinogenicity of aromatic amines involved *ortho* hydroxylation.[3] Thus, it seemed that **3,3′,5,5′-tetramethylbenzidine (TMB)** (in which *ortho* hydroxylation is impossible) might be an effective and safe substitute for benzidine. Indeed, it was found to be nontumorigenic by subcutaneous injection into rats.[4]

The *Salmonella*/microsome test (Ames test)[5] showed **TMB** to be nonmutagenic,[6,7] suggesting noncarcinogenicity. Recently, **TMB** was shown to be nonmutagenic when compared to other benzidine compounds in a modified Ames assay.[8]

Garner *et al.*[9] evaluated the use of **TMB** as a presumptive test for blood in forensic work. In various concentrations of glacial acetic acid, **TMB** reacted with blood in the presence of H_2O_2 to form a colored product. Comparative studies with benzidine showed **TMB** to be equally sensitive in blood detection.[9] Piejko and Boemer[10] have used **TMB** in a mixture spread on polyester film strips to determine glucose concentration in whole blood.

Standefer and Vanderjagt[11] found that methods employing **TMB** for plasma hemoglobin assay compared well with those using benzidine in accuracy, precision and sensitivity. Iron porphyrins, which can exhibit a peroxidase-like action, were detected at very low levels on paper chromatograms with **TMB** as the visualizing agent.[12] The peroxidase activity of the heme protein cytochrome P-450 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with **TMB** staining.[13] In this method, **TMB**-H_2O_2 stain is superior to benzidine-H_2O_2. The blue-stained bands are distinct and the color is stable.[13] Peroxidase activity was also detected by the presence of immunoprecipitates in Ouchterlony double-diffusion plates.[13]

In the area of neurohistochemistry, **TMB** proved more sensitive than 3,3′-diaminobenzidine in demonstrating retrograde and anterograde axonal transport of horseradish peroxidase (HRP) in rat brain by light microscopy.[14] **TMB** also gave results comparable to those obtained with benzidine dihydrochloride in the demonstration of retrograde transport of HRP.[15]

Current literature is replete with references to analytical applications of **TMB**. Recent applications include the use of **TMB** in a substrate to provide direct evidence of peroxidase activity in *Salmonella typhimurium*,[16] of horseradish peroxidase on virally infected plant root extracts,[17] and to measure the activity of peroxidase in an HRP-IgE complex.[18] **TMB** is gaining recognition as a chromogen in enzyme immunoassay systems.[18-20]

We also offer **TMB dihydrochloride** which, in contrast to the water-insoluble free base, dissolves slowly in water or in citrate buffer.[21] **TMB dihydrochloride** appears to be suitable for the quantitative determination of hemoglobin in solutions, and for the identification and localization of myeloperoxidase in cells.[21]

TMB and **TMB dihydrochloride** have earned acceptance as reliable and safe substitutes for benzidine and its carcinogenic derivatives.

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