which phenazine methosulphate is coupled to 2,6-dichlorophenol-indophenol. The pH optimum is 8.5 and the K_m for trimethylamine is about $2\mu\text{M}$. Of 42 compounds tried as substrate, the following gave an initial rate 10% or more of that with trimethylamine under the conditions tested: ethyldimethylamine, diethylmethylamine, 2-aminoethyldimethylamine, 2-hydroxyethyldimethylamine, 2-chloroethyldimethylamine and diethylamine.

The first step in the breakdown of trimethylamine by bacterium 4B6 thus appears to be the oxidative *N*-demethylation catalysed by trimethylamine dehydrogenase.

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The Metabolism of Trimethylamine *N*-Oxide by *Bacillus* PM6

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Bacillus PM6 is an aerobic spore-forming Gram-positive rod-shaped organism isolated from soil. It is capable of growth on trimethylamine *N*-oxide, trimethylamine, dimethylamine or methylamine as sole source of carbon, nitrogen and energy. Washed suspensions of trimethylamine *N*-oxide-grown organisms rapidly take up oxygen in the presence of trimethylamine *N*-oxide, dimethylamine and methylamine, but not in the presence of trimethylamine. Such suspensions, and their cell-free extracts, convert trimethylamine *N*-oxide anaerobically into dimethylamine and formaldehyde, indicating the presence of a trimethylamine *N*-oxide demethylase. Assays of trimethylamine *N*-oxide (Bystedt, Swenne & Aas, 1959), dimethylamine (by g.l.c. and paper chromatography) and formaldehyde (Nash, 1953) yield results that agree with the stoichiometry required by the following equation:

In early experiments the reaction rate was followed by measuring formaldehyde production colorimetrically (Nash, 1953); subsequently a more sensitive and convenient assay was developed in which the trimethylamine *N*-oxide demethylase is

coupled to formaldehyde dehydrogenase (EC 1.2.1.1) (partially purified from baker's yeast) and the reaction is followed spectrophotometrically at 340nm.

The trimethylamine *N*-oxide demethylase was purified 175-fold. The purified enzyme moves as a single protein on analytical polyacrylamide-gel electrophoresis and has a molecular weight of 50 000 as determined by gel filtration on Sephadex (Andrews, 1970). Treatment with 1% sodium dodecyl sulphate + 1% 2-mercaptoethanol caused no resolution of the protein as determined by polyacrylamide-gel electrophoresis (Weber & Osborn, 1969), suggesting that it consists of a single polypeptide chain; this experiment yielded a molecular weight of 37 000. Ultracentrifugal analysis (sedimentation equilibrium) gave a molecular weight of 36 000–47 000. The pH optimum of the purified enzyme is 7.5, the K_m for trimethylamine *N*-oxide is 2.85mM and the absorption spectrum shows only a single peak at 280nm. Ferrous iron, glutathione and L-ascorbate are strongly stimulatory.

Benzyltrimethylamine *N*-oxide, chlorpromazine *N*-oxide and (+)-propoxyphene *N*-oxide were also active as substrate for the enzyme, but the maximum rates obtained were only about 5% of that obtained with trimethylamine *N*-oxide. Trimethylamine is a non-competitive inhibitor, and SKF 525-A is a competitive inhibitor.

The initial step in the metabolism of trimethylamine *N*-oxide by *Bacillus* PM6 thus appears to be a demethylation, catalysed by trimethylamine *N*-oxide demethylase, yielding dimethylamine and formaldehyde.

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Configurational Dependencies of [^{19}F]Fluorine Chemical Shifts and Coupling Constants in Fluoromonosaccharides

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In general, chemical shifts and coupling constants for ^{19}F are of an order of magnitude greater

than values for ^1H (Elmsley, Feeney & Sutcliffe, 1966). Present studies are concerned with determining the values of these parameters for fluorine in distinctive configurations in a number of fluoromonosaccharides.

It has already been found that anomeric fluorine [F(1)] has a chemical shift (ϕ_c) of approx. 150 p.p.m. (with respect to trichlorofluoromethane) (Hall, Adamson, Johnson & Foster, 1970; Dwek, Kent, Kirby & Harrison, 1970) and that coupling with adjacent protons gives values of $J[\text{F}(1a)-\text{H}(2a)]$ 25 Hz and $J[\text{F}(1e)-\text{H}(2e)]$ 2 Hz. (Hall & Manville, 1967, 1968; Campbell, Dwek, Kent & Prout, 1969). Extended studies have shown that $-\text{CH}_2\text{F}$ in extracyclic positions, as in methyl 6-deoxy-6-fluoro- α -D-galactoside, has $\phi_c + 230$ p.p.m. similar to $-\text{CH}_2\text{F}$ in acyclic systems, e.g. fluoroacetamide where ϕ_c is +221.5 p.p.m. In contrast, $-\text{O}-\text{CF}_3$, as in an aglycone, has $\phi_c + 60$ p.p.m. The value of ϕ_c for F(2) is approx. 212 p.p.m. for a variety of fluoromonosaccharides, whereas F(3) in methyl 3-deoxy-3-fluoro- α -D-guloside has $\phi_c + 196$ p.p.m. The crystalline sugar reported as 3-deoxy-3-fluoro-D-glucose was found to give two peaks in g.l.c. (T_m 14.0 min and 16.8 min) in approximately equal amounts, and two ^{19}F shifts were observed of +194 and +200 p.p.m.

In the furanose series methyl 2,5-di-O-benzoyl-3-deoxy-3-fluoro- α -D-arabinoside has $\phi_c + 186$ p.p.m. whereas the corresponding 3-fluoroxylloside has $\phi_c + 202$ p.p.m. (in chloroform). Under high-resolution conditions the fluoroarabinoside showed a fine structure having $J[\text{F}(3)-\text{H}(3)]$ 46 Hz, $J[\text{F}(3)-\text{H}(2)]$ 7 Hz and $J[\text{F}(3)-\text{H}(4)]$ 10 Hz, and the xylo analogue had $J[\text{F}(3)-\text{H}(3)]$ 56 Hz, $J[\text{F}(3)-\text{H}(2)]$ 24 Hz and $J[\text{F}(3)-\text{H}(4)]$ 15 Hz. The *gem* F-H coupling constants of 53 Hz and 46 Hz for axial and equatorial fluorine respectively are similar to values reported for other positions in the ring (Hall & Manville, 1967; Hall *et al.* 1970).

Thus ^{19}F parameters, chemical shifts in particular, can provide significant stereochemical information about fluoromonosaccharides and open a way for the use of fluorine as a more general structural probe for biological macromolecules.

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N-Fluoroacetyl- α -D-glucosamine as a Molecular Probe of Lysozyme Structure by using [^{19}F]Fluorine Nuclear-Magnetic-Resonance Techniques

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In enzymic pathways involved in the biosynthesis of glycoproteins and mucopolysaccharides there is evidence that acylation of amino sugar intermediates is an important regulatory step (Kent, 1970). Chemical modification of such *N*-acyl groups, as in *N*-iodoacetyl- α -D-glucosamine, results in substantially altered biological properties, e.g. inhibition of glycoside transport and bacterial growth (Kent, Ackers & White, 1970; White & Kent, 1970; White, 1970). These properties appear to arise from the competitive occupancy by the haloacyl sugars of molecular sites specific for the glucose or *N*-acetylglucosamine configuration.

This hypothesis has been examined further on the basis of the close substrate analogy shown between metabolites and certain of their fluorinated derivatives (Kent, 1969). In particular, an investigation was made of the binding of *N*-fluoroacetyl- α -D-glucosamine by hen's-egg lysozyme, by using the large and distinctive ^{19}F chemical shifts that fluorocarbohydrates display (Dwek, Kent, Kirby & Harrison, 1970; Kent, Dwek & Taylor, 1971). *N*-Fluoroacetyl- α -D-glucosamine [m.p. 156–158°C, $[\alpha]_D^{25} + 29.4^\circ$ (c 1.0 in water, 3h); cf. Grieg & Leaback (1963): m.p. 189–192°C, $[\alpha]_D + 31^\circ$ (c 1 in water, 2h)], synthesized by dicyclohexylcarbodiimide condensation, has a ^{19}F chemical shift (ϕ_c) of +225 p.p.m. (with reference to trichlorofluoromethane at 33°C at 94 MHz in water or sodium acetate buffers between pH 5 and pH 7) comparable with extracyclic $-\text{CH}_2\text{F}$ [ϕ_c F(6) +230 p.p.m.]. At high resolution *N*-fluoroacetyl- α -D-glucosamine displays a 'deceptively simple' quartet of the ABX type as would arise from non-magnetic equivalence of the $-\text{CH}_2\text{F}$ protons.

In the presence of hen's-egg lysozyme in 50 mM-sodium acetate buffer, pH 7, *N*-fluoroacetyl- α -D-glucosamine shows a downfield shift of 13 Hz compared with enzyme-free controls. At high resolution the original quartet is observable with considerable band-broadening. Fluoroacetamide ($\phi_c + 221.5$ p.p.m.) gives its own distinctive triplet ($J[\text{F}-\text{H}]$ 53 Hz) when examined in the presence or absence of lysozyme; no change in chemical shift is observed in the presence of enzyme. Similarly no changes in ^{19}F n.m.r. parameters are observed with *N*-fluoroacetyl- α -D-glucosamine or fluoroacetamide in the presence of serum albumin. As will be reported elsewhere, haloacetylglucosamines are