positively charged substrate groups and EMA might prevent catalysis.

The stability of pepsin and EMA-pepsin in the pH-range above 6.0 was determined using calf chymosin as internal standard. The activity was measured relatively to pepsin at 30 °C. The results are given in Table 2 as half-lives. It is seen that the stability against the irreversible denaturation is three–five times better for EMA-pepsin than for unmodified pepsin.

For pepsin an electrostatic expansion of the negatively charged polypeptide chain may lead to denaturation at pH-values higher than 6. The enhanced stability of EMA-pepsin relative to pepsin may be attributed to different micro-environmental states of the two enzymes.

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Tritium—Protium Exchange in Dextran at 25 °C

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In the dextran (Sephadex®) gels the cross-links are connected by ether linkages to the anhydro-glucose residues of the dextran chains. Thus, at each point of attachment of a cross-link an hydroxyl hydrogen is lost and the concentration of hydroxyl groups remaining after cross-linking provides valuable information about the cross-linking structure. Since hydroxyl hydrogens will exchange with deuterium (D) or tritium (T)¹ provided they are accessible, tritium exchange can, thus, be used, giving information about the fraction of accessible groups, to determine the hydroxyl concentration.

The degree of accessibility of the Sephadex hydroxyls is, as yet, unknown. As regards other polysaccharides all the hydroxyls of amylose and starch, notwithstanding some degree of crystallinity in the latter, were reported to exchange with deuterium of deuterated water.² The 1,6-linked polysaccharides, e.g. dextran, are conformationally very flexible with an extended structure³ and inaccessibility is unlikely but should be tested for. The lack of crystallinity in a dry sample of the most highly cross-linked Sephadex gel,⁴ G-10, while not proof of accessibility, is strongly suggestive of it.

This communication reports an attempt to measure the tritium exchange constant of the parent polymer dextran as a model for the dextran gels. The exchange reaction between tritiated water (HTO) and the gel hydroxyls is

Because of the relatively large difference in their vibrational zero point energies,^{5,6} the bond dissociation energies of the different hydrogen isotopes differ sufficiently for the exchange constants to deviate considerably from unity in many systems; the T/H value usually diverging more than the D/H.

Experimental. Dextran® ($\bar{M}_w = 250\,000$; $\bar{M}_n = 113\,000$) was dissolved, without predrying, in double quartz-distilled water to a concentration of approximately 40 %. The concentration (weight fraction of dextran = 0.3604) was then calculated from the polarimetry⁷ (Na D line 589 nm) of triplicate gravimetric dilutions (~2 % solutions).

About 15 ml of the 36 % dextran solution were placed in one compartment of a double glass flange (FG 25, Quickfit, Great Britain) chamber and were separated from 15 ml water containing HTO (1 mCi/ml) in the other compartment by a hydrophobic polyvinylidene

fluoride 0.45 μm pore membrane (VF 6, GEL-MAN, Ann Arbor, Mich., U.S.A.).

The compartments were not stirred because of the high viscosity of the dextran solution. Kel-F grease (3 M, St. Paul, Minn., U.S.A.) was used to seal the membrane to the two flanges which were then held together by clamping the double chamber in a special holder which also served to support the chamber submerged in a water bath kept at $25 \pm 0.05^\circ\text{C}$.

This membrane has the useful property that although it allowed a rapid exchange of water molecules (the half-time for HTO or H_2^{18}O exchange was about 24 h) there was no net water transport for up to 15 days with concentration differences (sucrose) across the membrane of up to 0.2 M.⁸ As the dextran concentration used had an osmotic pressure corresponding to about 0.2 M,⁹ there should thus be no, or very little, net water transport. There was, in fact, a little, the dextran fraction decreasing from $f_d = 0.3604$ to $f_d = 0.3069$ after 13 weeks. This was due to influx of water from the "water" side since dextran was not detected chemically on the latter side at the end of the experiment.

Duplicate samples were taken from both compartments, suitably diluted (about 500 $\times$) and the tritium activities in ten 0.1 ml samples of the dilutions were measured. All samples and dilutions were weighed. The high dilutions ensured that the dextran concentration introduced into the scintillation fluid (Aquasol, NEN, Boston, U.S.A.) was not more than 0.006 %, a concentration¹ which did not affect the tritium counting rate significantly.

Samples taken after 3 weeks had not quite reached equilibrium (estimated > 95 %). Second samples were therefore taken 10 weeks later when equilibrium was assumed; the differences at 3 and 13 weeks were less than 5 %.

The exchange constant $K^{T/H}$ was calculated from the ratio (R) between the tritium activities in the two compartments, i.e.,

$$R = \frac{(3/162)K^{T/H}f_d + (2/18)(1 - f_d)}{(2/18)}$$

where f_d is the final dextran concentration and $K^{T/H}$ the T/H exchange constant; the figures refer to the numbers of exchange sites on the anhydroglucose residues (3) and water (2) and to the rounded molecular weights of the residues (162) and water (18).

The value of the exchange constant $\pm$ the estimated error of the estimate was,

$$K^{T/H} = 1.20 \pm 0.06.$$

The above constant is calculated assuming complete accessibility, which with regard to the structure of dextran and known properties of other related oligosaccharides seems reasonable.

Whether dextran is a satisfactory model for a cross-linked Sephadex is another question. In the 1,4- β -linked cellulose there is a marked ten-

dency for inter-chain coupling to occur when hydroxyls of different chains approach sufficiently closely to form hydrogen bonds¹⁰ which make the groups inaccessible to exchange. In Sephadex, particularly with the more highly cross-linked types where there may be considerable restriction in chain movement, this may also occur and requires further study.

Data on the T/H equilibrium exchange constant are apparently conflicting. In cellulose limited accessibility introduces considerable uncertainty into the calculation of the constant as indeed is the case with most biological macromolecules. Lang and Mason,¹ however, concluded that for cellulose the equilibrium T/H probably exceeded unity. In the open-chain peptides poly D,L-alanine and poly D,L-lysine, T/H and T/D were reported to be 1.21 and 1.09, respectively,¹¹ compared with the corresponding ratios in the solvent. The T/H ratios were also of a similar order in myoglobin¹² and oxidised ribonuclease¹³ although some uncertainty exists in these closed-chain peptides due to the question of accessibility. Lobunetz and Karush¹⁴ in a study of the distribution of tritium between gaseous ammonia and dry protein found that the latter was preferred.

The absence of a significant equilibrium isotope effect between tritium and deuterium has been reported in bovine plasma albumin¹⁵ and several other workers have found small or absent isotope effects.¹⁶

The problem in interpreting the data is much simpler in a polysaccharide such as dextran, where there is only one type of exchange group, and whose random conformation should promote accessibility. It is thus more comparable to the simpler homogeneous open-chain peptide than to a natural peptide constrained in a native protein and the close agreement of the $K^{T/H}$ value reported here with that of the open-chain peptides probably reflects this.

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Synthesis of 2,2',4,4',6'-Pentahydroxy-6-methylbenzophenone*

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2,2',4,4',6'-Pentahydroxy-6-methylbenzophenone (I) has often been postulated as a precursor of certain polyketides in lower plants, viz. xanthenes¹ and griseofulvin.^{1,2}

- I R=H
II R=CH₃
III R=CH₂C₆H₅

IV

For biosynthetic studies on lichen xanthenes (all of which are related to norlichexanthone,

* Part 33 of the series Chemical Studies on Lichens. Part 32 in press.

IV)³ I was needed. There appears, however, to be no record of its synthesis in the literature.

In an attempt to synthesize I, *O,O*-dimethylorsellinoyl chloride was reacted with 2,4,6-trimethoxyphenyl lithium in diethyl ether⁴ to give the methylated benzophenone II in ~50 % yield. During demethylation of II with boron tribromide in dichloromethane, I was formed (15 %) together with partially demethylated benzophenones and xanthenes.

In another synthesis, *O,O,O*-tribenzylphloroglucinol carboxylic acid was condensed with *O,O*-dibenzylorscinol using trifluoroacetic anhydride in dichloromethane⁵ to give the benzylated benzophenone III in high yield. 2,2',4,4'-Tetrabenzyl-6,6'-dimethylbenzophenone was formed as a by-product. Hydrogenolysis of III afforded I quantitatively.

The spectral data (NMR, UV, IR, MS) are in agreement with the pentahydroxy benzophenone I structure. (Cooling of the KBr disc prevented thermally induced formation of IV during the registering of the IR spectrum of I. When recording the mass spectrum of I, the main part of the sample dehydrated to IV in the direct inlet system before evaporation; however peaks at *m/e* 276, 151, and 153 were present).

The compound is sensitive to moisture even at room temperature. Other 2,2',6'-hydroxylated benzophenones are known to dehydrate fairly easily under alkaline conditions, even as the methyl ethers.²

Because of its great instability, it would be difficult to demonstrate the presence of I in plant material even if appreciable amounts should occur.

Experimental. All melting points are uncorrected. Elemental analyses were performed by the Analytical Department, Institute of Chemistry, Uppsala. All solvents used were of analytical grade. Petroleum ether refers to the fraction boiling at 43–55 °C. Column chromatography was done using Merck Silica Gel 60 (70–230 mesh); TLC was carried out using Merck's precoated silica gel plates. IR spectra were recorded with a Perkin-Elmer 157. NMR-spectra were recorded on a Varian A-60D, using TMS as the internal standard. UV-spectra were recorded on a Unicam SP 1800.

O,O-Dimethylorsellinoyl chloride.⁶ *O,O*-Dimethylorsellinic acid⁷ (7.2 g) in dry benzene (20 ml) was reacted with oxalyl chloride (3.5 ml) under nitrogen and left overnight at room temperature. Distillation (b.p. 123–128 °C/2 mmHg) gave 6.7 g (79 %) product which was extremely sensitive to moisture. NMR (CCl₄): δ 2.28 (s 3 H), 3.71 (s 3 H), 3.79 (s 3 H), 6.17 (s 2 H); IR ν_{max} (CCl₄) 1785 cm⁻¹ (C=O).

2,2',4,4',6'-Pentamethoxy-6-methylbenzophenone (II). 1,3,5-Trimethoxybenzene (3.60 g)⁸ in ether (25 ml) was treated with butyl lithium (14.3 ml, 16.7 mmol/ml in hexane) under nitrogen at room temperature (1 h). The slurry was slowly added to an ethereal solution (20