Table 3. The sparing effect of some nucleic acid components and amino acids on the requirement for PAB in *Rhodotorula* 18 b. AN-mixture: L-methionine 10 mg/l, DL-histidine 20 mg/l, L-leucine 10 mg/l, L-serine 10 mg/l, and thymine 30 mg/l.

Additions to the medium			Extinction value, Z, of the culture after	
PAB $\mu g/l$	AN-mixture + adenosine, 20 mg/l	AN-mixture + inosinic acid, 29 mg/l	104 h	144 h
0	—	—	250	350
1	—	—	290	400
10	—	—	450	660
50	—	—	710	1070
100	—	—	870	1260
0	+	—	300	370
1	+	—	520	680
10	+	—	700	970
50	+	—	870	1300
100	+	—	840	1290
0	—	+	330	450
1	—	+	480	690
10	—	+	570	880
50	—	+	880	1380
100	—	+	820	1320

Rodriguez [9]). Preliminary tests with thymine, amino acids and various purines, alone and combined, showed that a mixture of adenosine, thymine and four amino acids gave the highest rate of growth in the presence of PAB (Table 3). This mixture was not a substitute for PAB, but exerted a sparing action, so that half maximum growth rate was attained with approximately 1 μg instead of 10 μg of PAB per liter. Adenosine could be exchanged for inosinic acid with no appreciable loss of activity of the mixture.

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Observation of Proton Magnetic Resonance Shifts in H_2O-D_2O Mixtures

MATS S. BERGQVIST* and L. E. GÖRAN ERIKSSON

Division of Physical Chemistry, The Royal Institute of Technology, Stockholm 70, Sweden

Isotope effects on nuclear magnetic resonance have been reported for specific groups in organic compounds. Carbon and hydrogen isotope effects have been observed in fluorine and proton magnetic resonance (PMR). In all the investigations the resonance of observed nuclei in a group containing a heavy isotope of the same or another element was displaced upfield relative to the resonance in a group with a light isotope. The present note reports a PMR study of $H_2O - D_2O$ mixtures. PMR shifts were determined at 60 Mc/sec both by internal and external reference methods. Recently the PMR spectrum of the HDO and H_2O species in an acetone solution was reported [1]; the HDO resonance signal, a triplet due to the H − D spin-spin coupling, was observed at a slightly higher field than the singlet line of H_2O.

Experimental. The PMR spectra were recorded with a Varian Associates A-60 spectrometer, operating at the fixed frequency of 60 Mc/sec. The resonance line shifts were determined by means of side band technique using a Hewlett Packard Model 202 A audio oscillator. The determination of the frequency was carried out by means of a Model 5512 A electronic

* Present address: Sandvikens Jernverks AB, Sandviken, Sweden.

counter of the same manufacture. The oscillator frequency was observed as the sweep passed the first side band signal. The value given by the frequency counter is the mean oscillator frequency. The procedure was repeated several times and the shifts obtained were averaged.

The deuterium oxide was 99.8 % ($d_4^{20} =$ = 1.10518) from Norsk Hydro, Norway. Ordinary water was distilled in a quartz apparatus. No precautions to keep the water deaerated were taken since dissolved oxygen has no observable influence on the resonance shifts [2]. The mixtures were prepared by weighing. 0.3 m t-butanol was used as internal standard which is convenient in aqueous solution [3]. As external reference, benzene was placed in the annular space between precision bore coaxial glass tubings. The temperature in the probe was 32.0 ± 0.1°C. The spinning samples were kept in the probe long enough to obtain that temperature. The influence of a small temperature drift was taken into consideration by measuring the samples in a random order. As there is an enrichment of ^{18}O in deuterium oxide the shift in water enriched in ^{18}O from Fluka A. G. was also measured.

Results. The experimental resonance shifts, all upfield relative to H_2O, in mixtures of different mole fractions of D_2O are plotted in Fig. 1. The proton resonance in pure H_2O has been taken as zero. Values obtained with external reference have been corrected for the macroscopic magnetic susceptibility shifts, the susceptibility being a strictly additive function of the concentration [4]. The correction at 60 Mc/sec for a cylindrical specimen is [5]

$$60 \times 10^6 \times (2\pi/3)\{d[y\varkappa_D + (1-y)\varkappa_H] - \varkappa_H d_H\} \text{ c/sec}$$

where y is the weight fraction of D_2O, $\varkappa_D$ and $\varkappa_H$ are the specific susceptibilities of D_2O and H_2O, respectively. The values -0.6467×10^{-6} and -0.7200×10^{-6} at 20°C were chosen as the most accurate available [4], and the temperature dependence of the specific susceptibilities may be neglected. In this formula d is the density of the mixture calculated under the assumption of additive molar volumes [4]. The density of H_2O is denoted by d_H. The maximum calculated susceptibility correction, *viz.* that of D_2O, was -0.40 c/sec at 60 Mc/sec.

In Fig. 1 straight lines have been fitted to the measured points according to the method of least squares. When the temperature was carefully adjusted (by means of the spinner turbine air stream) it was pos-
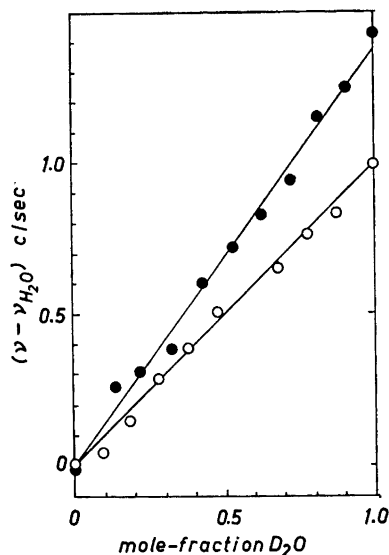
Fig. 1. PMR shift in $H_2O - D_2O$ mixtures *vs.* concentration of D_2O at 32°C. ○ Internal reference; ● external reference.

sible to reproduce the straight lines within the standard deviation of the first experimental series. The inclination of the line of the internal reference method was 1.00 c/sec/mole fraction D_2O, as compared to 1.38 c/sec/mole fraction D_2O of the external reference method. Standard errors in both cases were ± 0.04 c/sec/mole fraction D_2O. The value of the inclination is identical with the PMR shift between D_2O and H_2O. In a water specimen enriched to 5 % in ^{18}O a small, not significant, upfield PMR shift relative to ordinary water was observed. Since this enrichment is several times larger than that expected in concentrated D_2O no corrections due to the ^{18}O content were needed. The effect of an enrichment in ^{17}O (spin 5/2) has not been studied.

Discussion. The PMR shift in $H_2O - D_2O$ mixtures appears to be a linear function of the deuterium concentration. The results obtained by the internal and external reference methods do not coincide. The following reasons for the deviation may be stated: (*a*) erroneous susceptibility corrections, (*b*) temperature instability effects, (*c*) internal standard effects and (*d*) nuclear relaxation effects. The corrections for the macroscopic susceptibility may be incor-

rect either because the applied theoretical formula is too approximate or due to errors in densities or specific susceptibilities. The shifts are very sensitive to temperature instability and in a separate investigation with a Varian Associates V-4300 B spectrometer, operating at 40 Mc/sec, a temperature dependence was observed in the range $0-70°C$. It was found that the shift between a water specimen and external benzene reference satisfied the equation

$$(H_{H_2O} - H_{C_6H_6})/H_{C_6H_6} = 8.828 \times 10^{-3}\ t + 1.527\ \text{ppm}$$

where H is the resonant field and t is the temperature in °C. The method of least squares was applied to obtain the constants of the equation. Accordingly, the temperature coefficient for the shift between water and external benzene is 0.53 c/sec, °C at 60 Mc/sec. It was, however, possible to reproduce the lines from time to time and thus temperature instability as a reason for the deviation may be discounted. Due to anomalies expected at low internal standard concentrations it is not possible to eliminate the effect of the standard by extrapolating to zero concentration. The alcohol will take part in the hydrogen transfer and also break hydrogen bonds in the water lattice. In H_2O and D_2O these interferences may be of different magnitude. It is not likely that the effects will influence the chemical shift of the reference methyl protons very much. The connection between different relaxation mechanisms and observed shifts are far from clear. Without further experimental information it seems, however, difficult to settle the question of the observed discrepancy between the internal and external reference methods.

The heavier deuteron isotope slows down the rapid hydrogen transfer in D_2O as compared to H_2O. Accordingly, the mean life time of a preferred hydrogen position in the neighbourhood of the one oxygen of the two in a hydrogen bond will increase, anp then upfield shifts ("increased shieldings") are originated. Moreover, the difference in the strength of the deuterium and protium hydrogen bond must be noticed. A linear decrease of the self-diffusion coefficient with increasing deuterium concentration was found in H_2O-D_2O mixtures [6]. This is in agreement with our result on the PMR dependence. In a diluted acetone solution the HDO triplet was centered at 0.03 ppm toward higher field relative to the H_2O line [1]. The temperature was not reported. Our results indicate a PMR shift for a proton in D_2O relative to that in H_2O of about 0.02 ppm (average between internal and external reference methods). The lower value is probably due to the rapid hydrogen transfer in absence of organic solvent, resulting in a decreased resonant field for the proton.

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