TABLE II. $[(C_6H_5)_3P]_3RhCl$ Catalyzed Hydrosilation of α,β-Unsaturated Esters[5]

Unsaturated ester	Silane	Temp. °C (min)	% yield of keten trialkylsilyl methyl acetal[a]	% yield of 1,2-addition product
CH_3–CH=CH–COOMe	Me_3SiH	60(10)	77.0	8.6 (α-silyl)
	Et_2SiH	100(1)	85.6	—
	n-Pr_3SiH	100(1)	73.5[b,c]	—
C_2H_5–CH=CH–COOMe	Et_3SiH	100(1)	75.4	—
iso-C_3H_7–CH=CH–COOMe	Et_3SiH	100(1)	76.5	—
CH_2=C(Me)COOMe	Et_3SiH	100(1)	70.0[b,d]	—
CH_2=CH–COOMe	Et_3SiH	100(1)	31.6	38.9 (β-silyl)

a) The yields are based on saturated esters after methanolysis except noted. Geometrical isomerism of keten acetals was not determined.
b) isolated yield obtained by short-path distillation
c) bp 74° (1 mmHg); IR $\nu_{C=C}$: 1678 cm^{-1}
d) bp 38—40° (1 mmHg); IR $\nu_{C=C}$: 1705 cm^{-1}

chlororhodium, on the other hand, was found to be the catalyst of chloice for the preparation of keten silyl acetals from α,β-unsaturated esters, particularly under combination with triethylsilane.

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Enhancement of the Fluorescence Intensity of Dansyl Protamine in the Presence of Sulfated Carbohydrates and Its Application to Assay of Mucopolysaccharides and Synthetic Sulfates of Mono-, Oligo-, and Polysaccharides

Determination of naturally occuring mucopolysaccharides (MPS) and synthetic sulfates of carbohydrates are currently of much biological and clinical importance.[1] Microamount of these saccharides has hitherto been assayed by the use of metachromasy of dyes[2] and enzymatic degradation.[3] However, metachromasy is largely affected by pH, temperature, and ionic strength of the reaction mixture. Moreover, the reagent blank shows large absorbance in this method. On the other hand, enzymatic method is only applicable to limited number of MPS and not applicable to synthetic sulfates. In the present study, protamine, which has been demonstrated to bind tightly with heparin and other mucopolysaccharides,[4] was labeled with

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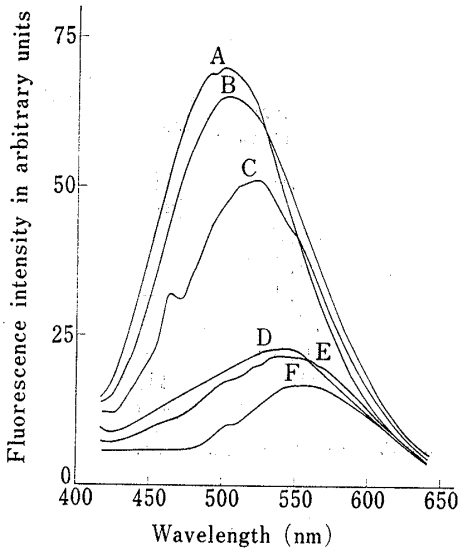

Fig. 1. Fluorescence Spectra of 14 μg/ml of DNSP in the Presence of Sucrose sulfate (A), cellulose sulfate (B), heparin (C), chondroitin sulfate A (D), and hyaluronic acid (E). F indicates the spectrum of DNSP (14 μg/ml) alone.

1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS-Cl). The fluorescence intensity of the product, DNS-protamine (DNSP), was found to be greatly enhanced by addition of MPS and other sulfated mono-, oligo-, and polysaccharides. This finding was successfully applied to the assay of these carbohydrates.

DNS-protamine was prepared using cycloheptaamylose-DNS-Cl complex as previously described.[5] Degree of substitution in mol of DNS group per mol of protein was 0.72 as determined by measuring absorbance at 334 nm and estimating protein by the Lowry procedure.[6] Figure 1 displays the fluorescence spectra of DNSP in the presence of several sulfated carbohydrates. Synthetic sulfates of carbohydrates induced greater fluorescence than natuarlly occuring MPS. We have previously reported that the association constants for binding of these synthetic sulfates with bovine serum albumin were larger than those for MPS.[7] Among the MPS tested, heparin gave the largest increment of fluorescence. Hyaluronic acid, which carries no sulfate group, showed only small enhancement.

Maximum fluorescence was observed at the pH in the range of 8.5 to 9.5. This sulfate-induced fluorescence was almost completely quenched by addition of 1M KCl and 7M urea indicating electrostatic and hydrophobic interactions.

The assay procedure for the sulfated carbohydrates is as described below. To 1 ml of sample solution is added 3 ml of 14 μg/ml DNSP in 50 mM carbonate buffer, pH 9.3, and the fluorescence intensity was measured at excitation and emission wavelength of 365 and 525 nm, respectively, at 25°. The standard curve for heparin was liner in the range of final concentration of 0.5 to 10 μg. The fluorescence intensity of 10 μg/ml of heparin was not affected in the presence of 250 μg/ml of urea, potassium chloride, glucose, glycine, alanine, phenylalanine, bovine serum albumin, sodium acetate, ammonium sulfamate, dextran, and starch. This procedure is simple, rapid, and sensitive. The fluorescence of the reagent blank is low because the fluorescence of DNSP is largely quenched in aqueous media. This method is expected to be useful in the assay of a large numbers of samples at one time, such as eluates from chromatographic column, or in the rapid scanning of clinical samples.

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