Thermotropic Behavior of Cholesterol-Linked Polysaccharides

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Received 21 August 1997; accepted 24 March 1998

ABSTRACT: Cholesterol-linked polysaccharides were prepared by reacting monocholesterylsuccinate (ChMS) with cellulose acetate [AC; degree of substitution (DS) 1.00, 1.80, and 2.33], ethyl cellulose (EC; DS 2.46), starch, and hydroxyethyl starch (HES; molar substitution, 0.05–0.07). The degree of ChMS substitution ranged from 0.27 to 1.29. The polymers were characterized by IR, NMR, DSC, and hot-stage coupled polarizing microscopy. Polymers with a higher DS of ChMS showed a thermotropic liquid crystalline behavior. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 195–201, 1998

Key words: thermotropic; polysaccharides; cholesterol; cellulosics; polarizing optical microscopy

INTRODUCTION

Cellulosic polymers display liquid crystalline properties. The first cellulose derivative reported to exhibit lyotropic liquid crystalline behavior was hydroxypropyl cellulose.[1] Several reviews on cellulosic liquid crystals have appeared.[2–4] Hydroxypropyl cellulose[5] and its derivatives[6–8] have been shown to be thermotropic materials. Recently, mesophase formation in hydroxyethyl cellulose acetate[9] and fully decanoated cellulose[10] was reported. Tri-*o*-benzylcellulose, prepared in a homogeneous medium, with a $\overline{DP}w$ of 130 showed characteristics of a thermotropic liquid crystal.[11]

In all these investigations, the substituents introduced onto the cellulose backbone are nonmesogenic. Here, we report on the synthesis and mesomorphic behavior of monocholesterylsuccinate derivatives of acetyl cellulose (AC), ethyl cellulose (EC), starch, and hydroxyethyl starch (HES). The effect of the degree of substitution (DS) on the mesomorphic behavior of a monocholesterylsuccinate derivative of hydroxyethyl cellulose forms the basis of another communication. Cholesterol is important in the field of liquid crystals and biological materials. Side-chain liquid crystalline polymers where cholesterol is used as mesogenic units have been reported.[12–14] It is not necessary that the mesogenic group is present at every repeating unit as is indicated by the realization of a mesophase in the copolymer of the cholesteryl monomer with nonmesogenic monomers.[14] Combined liquid crystalline polymers containing mesogenic units in both the main chain and side chains with the side chains attached to rigid rodlike polymer main chain are known.[15] This suggests that introducing a bulky substituent onto a semirigid backbone would not necessarily preclude mesophase formation. The intended application for the monocholesterylsuccinate derivatives of polysaccharide is as bile acid/cholesterol sequestrants. Many studies describe the use of a variety of carbohydrate-derived materials including hydroxypropyl methyl cellulose

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Contract grant sponsor: Department of Science and Technology (DST), New Delhi; contract grant number: SP/S1/G-72/90.

Journal of Applied Polymer Science, Vol. 70, 195–201 (1998)

 CCC 0021-8995/98/010195-07

for treating high blood cholesterol levels.[16] The polysaccharides lower the cholesterol level by functioning as viscosity builders in the intestine, where they reduce translational diffusion, lowering the absorption of all food ingredients, including cholesterol and bile acid. There may be ionic interactions with sterols, thereby eliminating them from circulation. We feel that cholesterol-linked polysaccharides capable of mesophase formation would have enhanced bile acid/cholesterol sequestration, probably through mixed mesophase formation with the free bile acids/cholesterol in the intestine.

EXPERIMENTAL

Materials

Ethyl cellulose (EC; Aldrich, Hyderabad, India), starch (BDH Chemicals, London, UK), and cellulose acetate (CA; DS 2.55 Loba Chemie, Mumbai) were purified by Soxhlet extraction before use. Hydroxyethyl starch (HES; molar substitution, MS 0.05–0.07) was a gift sample from the Anil Starch Products Ltd. (Ahmadabad, India). Other reagents and solvents purchased from commercial sources were purified before use. Succinic anhydride was prepared as per the reported procedure.[17]

Preparation of Monocholesterylsuccinate (ChMS)

Cholesterol, 25 g (0.0646*M*), was dissolved in 60 mL of pyridine and succinic anhydride, 18 g (0.18*M*), was added to it. The reaction was allowed to proceed at 90°C for 24 h under stirring. At the end of 24 h, the dark brown-colored reaction mixture was cooled to room temperature and precipitated into distilled water. The preciptate was extensively washed with distilled water. The product obtained was first air-dried and then purified by recrystallization in acetone. A recrystallized product was then dried under a vacuum at room temperature. The reaction was carried out under a nitrogen atmosphere. Yield, 28 g, 89%; mp 185°C.

Hydrolysis of CA

CA with a DS of 2.55 was hydrolyzed in acidic medium as reported earlier to obtain CA of a lower DS. The hydrolysis was carried out for 48 h at room temperature and for 72 h at 40°C in the presence of hydrochloric acid to obtain CA with a DS of 1.8 and 1.0, respectively.

Preparation of ChMS Derivative of Polysaccharides

A predetermined amount of ChMS was placed in a three-necked round-bottomed flask equipped with a reflux condenser, calcium chloride guard tube, and magnetic stirrer. Dry and distilled hexane (30 mL) was added to the flask followed by the addition of an excess of thionyl chloride. The reaction mixture was stirred at room temperature for 24 h. The excess of thionyl chloride and the solvent was removed under reduced pressure. Benzene (20 mL) was added to the reaction flask and the contents stirred for a few minutes. Benzene was then removed under reduced pressure. The addition and removal of benzene was repeated three times to ensure complete removal of the unreacted thionyl chloride.

The contents of the flask were further dried under a vacuum to near dryness and dry, distilled dimethylacetamide (DMAc, 10 mL) was introduced into the flask. When a clear solution resulted, it was transferred to another flask containing the desired amount of the polysaccharide and DMAc (10 mL). The reaction was allowed to proceed at room temperature or at 70°C for 15–96 h depending upon the polysaccharide used. At the end of the reaction, the reaction mixture was poured into an excess of methanol. The precipitate was washed several times with methanol, filtered, and dried. The air-dried product was Soxhlet-extracted with methanol to ensure the complete removal of unreacted ChMS.

The starch–ChMS reaction product was further Soxhlet-extracted with benzene to separate into two fractions. Both the benzene-soluble and benzene-insoluble fractions were used in the present work.

GENERAL ANALYSIS

IR spectra of the products were recorded on Perkin–Elmer 883 spectrometer using the nujol mull or KBr pellet technique. Proton NMR spectra of ChMS was recorded in $CDCl_3$ with tetramethylsilane as an internal standard on a Brucker 200-MHz spectrometer. The thermal properties of the polymer were studied by polarizing light microscopy on a Leitz Laborlux 11 POL S polarizing optical microscope (POM) equipped with a Leitz Wetzlar microscope heating stage 350 and a Wild

Table I Reaction Conditions for Preparation of ChMS Derivatives of Polysaccharides

Sample	Parent Polymer[a]	ChMS (g)	Temperature (°C)	Time (h)	DS of ChMS in Product
ACChMS 100	AC (acetyl DS 1.0)	7.13	RT	96	1.29[b]
ACChMS 180	AC (acetyl DS 1.8)	3.33	RT	72	0.57[b]
ACChMS 233	AC (acetyl DS 2.33)	3.33	RT	72	0.27[b]
ECChMS	EC (ethyl DS 2.46)	1.67	RT	15	0.27[c]
SChMS I	Starch	2.5	70	24	0.36[c]
SChMS II[d]	—	—	—	—	1.18[c]
HESChMS	HES (hydroxyethyl MS 0.05)	3.5	70	48	0.69[c]

RT, room temperature.
[a] Parent polymer 1.0 g.
[b] Calculated from weight increase.
[c] Determined by alkaline hydrolysis.
[d] Isolated from SChMS I.

MPS 0.32× camera. DSC analysis was carried out on a Mettler TA 4000 series instrument. It consisted of a DSC 30 cell coupled to a Mettler TC 11 TA processor. The heating and cooling rate was 10 K/min.

The DS was quantitatively determined by saponification. In a typical run, about 0.3 g of the dried polymer was dissolved in 20 mL of pyridine and then 20 mL of standard NaOH was added. The hydrolysis was carried out for 1 h at the reflux temperature. After cooling it to room temperature, the excess of alkali was backtitrated conductometrically with standard hydrochloric acid. A blank titration was carried out in a similar manner using the corresponding polysaccharide and cholesterol in a weight ratio nearly the same as that of the product which was estimated from the weight increase.

RESULTS AND DISCUSSION

The structure of ChMS was confirmed by elemental analysis and IR and proton NMR spectroscopy, the details of which will be reported separately. ChMS was converted into its acid chloride and subsequently reacted with AC, EC, starch, and HES to obtain ChMS derivatives of the polysaccharides recorded in Table I. The reaction conditions were chosen arbitrarily. The weight ratio of the ChMS/parent polymer was higher when the parent polymer contained more free hydroxyl groups per anhydroglucose ring and the reaction was carried out at lower temperature. Reaction time was longer when the parent polymer was not completely soluble. The degree of ChMS substitution was determined by alkaline hydrolysis of the samples with an excess of standard alkali and backtitrating the excess of alkali with standard acid. The combined degree of acetyl/ethyl and ChMS substitution was found to be higher when the DS of the initial substituent was higher, being 2.29, 2.37, 2.60, and 2.71 for ACChMS100, ACChMS180, ACChMS233, and ECChMS, respectively. The percentage of free hydroxyls reacted is in the range of 40% for ACChMS233 to about 64% for ACChMS100. Compared to these products, the reaction with starch and HES did not proceed as well.

IR spectra of the polymers are shown in Figure 1. The IR spectrum of ECChMS shows a substantial decrease in the intensity of the hydroxyl-stretching bands appearing at about 3350–3500 cm^{-1}. A peak due to >C═O stretching vibrations is seen at 1726 cm^{-1}, which confirms the esterification. A significant increase in the intensity of $–CH_2—$ stretching vibrations at about 2880–3050 cm^{-1} is due to the large number of methylene groups of the ChMS moiety.

ACChMS100, ACChMS180, and ACChMS233 are AC-based polymers which already possess ester linkages. However, a comparison of their IR spectra reveals that as the combined DS of acetyl and ChMS substituents increases from 2.29 for ACChMS100 to 2.37 for ACChMS180 to 2.60 for ACChMS233 the intensity of the hydroxyl

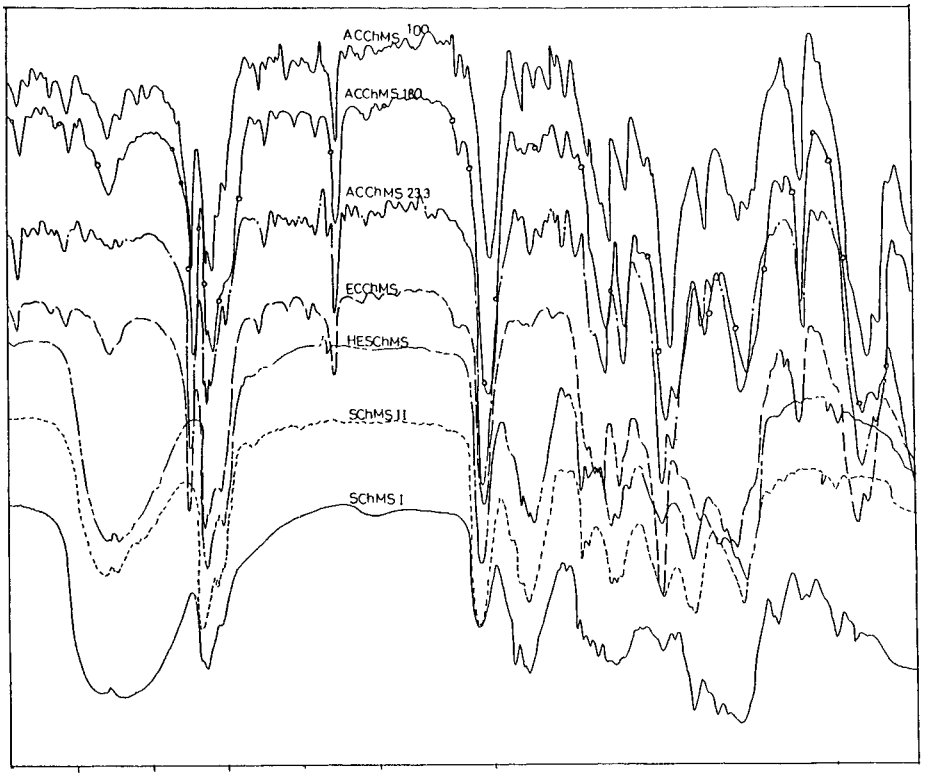

Figure 1 Infrared spectra of cholesterol-linked polysaccharides.

stretching vibration at 3400–3500 cm^{-1} decreases while that of the —CH_2— and >C═O stretching vibrations at around 2900 and 1740–1750 cm^{-1}, respectively, increases. As in the case of ECChMS, SChMS and HESChMS revealed a >C═O stretching band at around 1740 cm^{-1}, confirming esterification. They also show a strong peak at around 3400–3500 cm^{-1} due to hydroxyl stretching, supporting the earlier inference that the esterification reaction did not proceed too well in these cases.

NMR spectra of the polymers together with that of ChMS are shown in Figure 2. A multiplet at 2.65 ppm seen in the NMR spectrum of ChMS may be attributed to the two adjacent methylene groups of the succinoyl moiety. Other peak assignments were similar to those reported earlier.[18] Although proton NMR has been utilized extensively for compositional analysis as well as for structural characterization of polymers, its application to industrial cellulosic polymers has been rather limited due to the relatively low solubility and high viscosity of the solutions of these materials. This is evident from the NMR spectra of SChMS, HESChMS, and ECChMS, which are poorly resolved. However, there are discernible peaks in these spectra at 3.0–4.5 ppm. To confirm this, the spectrum of ECChMS was expanded along the *Y* axis, which confirmed the presence of signals from the glucopyranose ring. The NMR spectrum of ACChMS233 displays proton signals from the cellulose backbone as well as those from the ChMS. The peak for the two adjacent methylene groups of ChMS appears at 2.65 ppm. But it is poorly resolved compared to the one in the ChMS spectrum. The glucopyranose ring proton signals are seen at 3.0–5.2 ppm.

The DSC thermograms of ACChMS180 and ACChMS233 obtained in the heating mode showed a single endothermic peak at 221.2° and 230°C, respectively. ECChMS revealed a broad endothermic peak over 150–220°C. The peaks correspond to a crystal-to-isotropic fluid transition as observed under a polarizing light microscope. No distinct thermal transitions were observed in the cooling mode for these polymers and in both heating and cooling modes for ACChMS100, SChMSII, and HESChMS.

All the polymers were slightly yellowish nonbirefringent powders at room temperature. They were placed on the hot stage of the microscope and heated at a rate of 10°C/min. ACChMS100

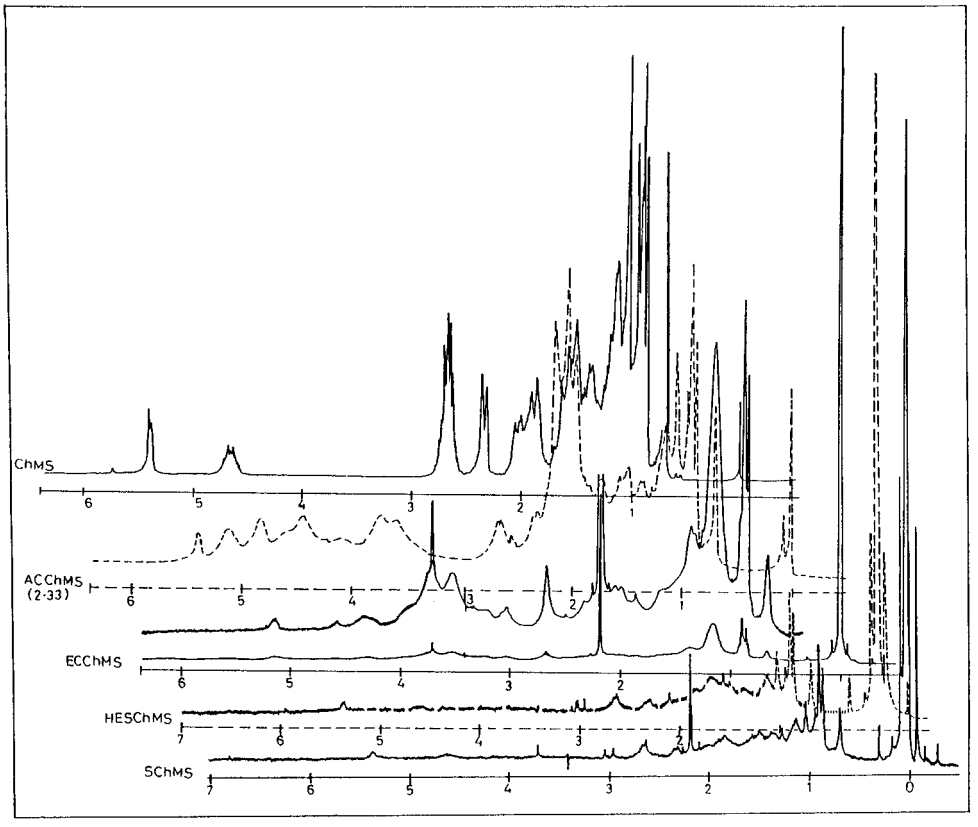

Figure 2 Proton NMR spectra of cholesterol-linked polysaccharides.

softened above 100°C. This was checked by applying slight pressure on the coverglass. When pressed slightly, the material showed birefringence which disappeared immediately after releasing the pressure. Melting began at about 170°C and was complete at about 195°C. No birefringence was observed during heating unless the material was pressed above 100°C and before melting began. The molten sample was isotropic. On cooling at the same rate, very little birefringence was observed from below 100°C to room temperature. The sample was reheated until it melts at 185°C, at a temperature lower than the one observed during the first heating. The isotropic nonbirefringent melt was cooled to 100°C when very little birefringence was observed. It was held at this temperature for 30 h, while checking intermittently. An increase in the birefringence was observed after 22 h until the whole viewfield became birefringent, displaying what appeared to be a batonnetlike texture shown in Figure 3. There was no change in the texture upon further standing up to 30 h at the same temperature. The birefringence was retained at room temperature. Heating and cooling was repeated twice with the same observation.

ACChMS180 and ACChMS233 did not show birefringence either on heating or cooling. On heating, ACChMS180 showed softening above 130°C as indicated by the appearance of birefringence when the sample was pressed slightly. The birefringence disappeared quickly when the pressure was released. Melting was observed at 217–220°C, which coincides with the endotherm peak found in the DSC thermogram. The melt was completely isotropic. It was further heated up to 230°C and then cooled. No birefringence was observed on cooling up to room temperature. Annealing at 180°C for 24 h did not result in the development of birefringence. On the other hand, the sample turned somewhat dark brown, showing the signs of decomposition. Similar observations were made with the ACChMS233. It showed softening above 180°C and began to melt at 220°C and the melting was complete at 230°C. The melting point values of ACChMS188 and ACChMS233 determined using the hot-stage microscope coincided with those determined from the corresponding DSC thermograms.

A comparison of the softening and melting temperatures of ACChMS180, and ACChMS233, which have a common parent polymer, reveal that

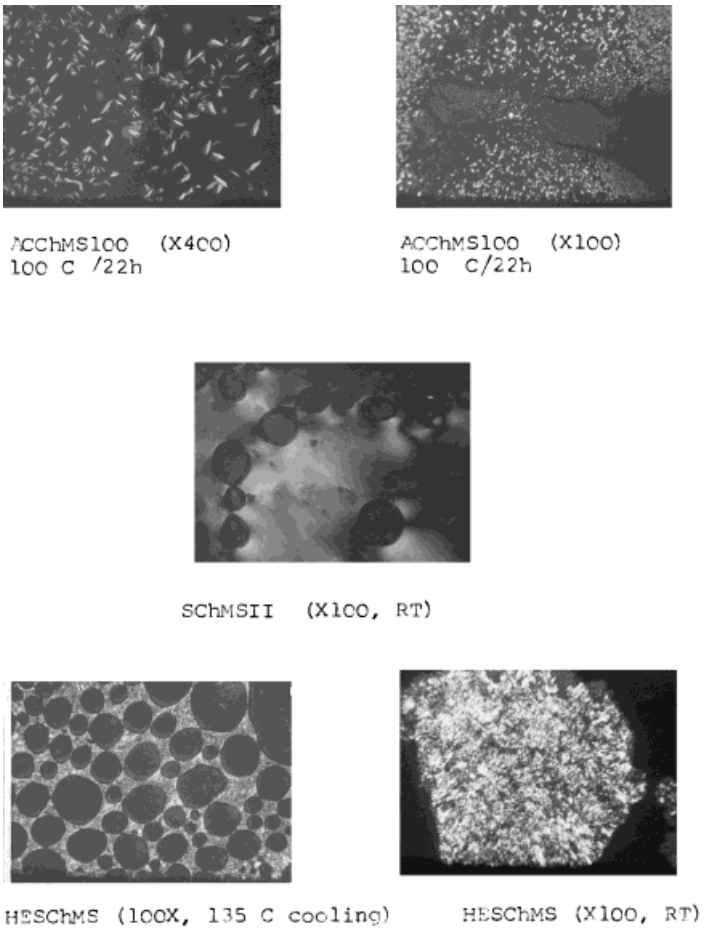

Figure 3 Photomicrograph (crossed polars) of ACChMS 100, SChMS II, and HES-ChMS.

as the degree of ChMS substitution increases from 0.27 for ACChMS233 to 0.57 for AC-ChMS180 to 1.29 for ACChMS100 the melting temperature decreases from 230 to 220 to 195°C, respectively. The softening temperature is even more affected, decreasing from 180°C for AC-ChMS233 to 130°C for ACChMS180 to 100°C for ACChMS100. It is interesting to note that the combined DS of acetyl and ChMS substituents is highest in the case of ACChMS233 and lowest in the case of ACChMS100. Even though the combined DS for ACChMS 100 and ACChMS 180 is nearly equal ($\sim$ 2.30), there is a great difference in their thermotropic behavior which may be attributed to the higher DS of the bulky, mesogenic ChMS.

Ethyl cellulose with a DS of 2.5 has been shown to exhibit thermotropic liquid crystalline behavior.[19] ECChMS, where the parent polymer is EC, did not reveal mesophase formation. Melting occurred over a temperature range from 150 to 220°C. SChMS was separated into two, benzene-soluble and benzene-insoluble, fractions. SChMS I, the benzene-insoluble fraction, began to melt above 205°C and the melting was complete at about 230°C. No birefringence was observed during heating or on cooling up to room temperature.

SChMSII, the benzene-soluble fraction, melted completely at 145°C. No birefringence was observed during heating. The isotropic melt was further heated up to 160°C and cooled. Birefringence appeared at 100°C and was retained at room temperature (Fig. 3). The difference in the thermal behavior of SChMS may be attributed to its degree of ChMS substitution.

For HESChMS, birefringence was observed, on pressing the coverglass slightly, above 140°C. As opposed to other samples, it remained birefringent when the pressure was released. Birefringence increased with increasing temperature up

to 190°C and then decreased over a broad temperature range and disappeared completely at 210°C to give an isotropic phase. On cooling, the birefringence appeared at 175°C, which was retained at room temperature. On reheating, batonnets were observed just before isotropization at 190°C, and again on cooling the isotropic melt from 190°C, batonnets were observed at 165°C, which quickly coalesced into an unidentifiable texture (Fig. 3).

CONCLUSIONS

The reaction of ChMS with various polysaccharides yielded cholesterol-linked polysaccharides with a ChMS DS ranging from 0.27 to 1.29. Polymers with a higher DS, namely, ACChMS100 (DS 1.29), SChMS (DS 1.18), and HESChMS (DS 0.69), showed mesomorphic behavior as against those with a lower ChMS DS. The mesomorphic behavior, though at present, cannot be conclusively attributed either to the semirigid backbone of the polymer chain or mesogenic substituent groups, the fact that ACChMS233 with a combined DS (2.6) greater than that of ACChMS100 (combined DS 2.29) does not show mesomorphic behavior indicates that it may be due to the mesogenic substituent groups. It is evident from the above results that the semirigid backbone polysaccharides when substituted with the mesogenic moiety are capable of the formation of a mesophase.

The authors (V. A. E. S., N. N. M., and S. V. L.) are grateful to the Department of Science and Technology (DST), New Delhi, for the financial assistance through Grant No. SP/S1/G-72/90.

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