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A NEW FACILE METHYLATION METHOD FOR CELL-WALL POLYSACCHARIDES

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ABSTRACT

A new methylation method involving powdered sodium hydroxide and methyl iodide has been developed for the facile methylation of cell-wall polysaccharides. Commercial cellulose powder, wood cellulose, and unbleached kraft pulp in solution in SO_2–diethylamine–methyl sulfoxide could be completely methylated. Suspensions of holocelluloses, prepared from spruce and beech wood-meals and containing <5% of lignin, and cell-wall polysaccharides containing relatively large amounts of uronic acid and isolated from midrib of *Nicotiana tabacum* (CWM), were almost completely methylated in one step. Some decarboxylation occurred with the latter polymers.

INTRODUCTION

Methylation analysis of oligo- and poly-saccharides now frequently involves the Hakomori method[1]. However, this method has some defects. Thus, the preparation of sodium methylsulfinylmethanide in methyl sulfoxide is troublesome and the reagent is not stable, depolymerisation of polysaccharides cannot be avoided, and polysaccharides that are soluble in methyl sulfoxide sometimes resist complete methylation in one step and repetition of methylation can cause severe depolymerisation.

Some modifications of the Hakomori method and new methylation methods have been reported. The use of potassium hydride[2] or potassium *tert*-butoxide[3] in place of sodium hydride in the Hakomori method has been proposed. The former proved to be beneficial, but not the latter[4]. Cell-wall polysaccharides and cellulosic materials insoluble in methyl sulfoxide have been methylated by a modification[5] of the Hakomori method, but complete methylation cannot be attained in one step. Narui *et al.*[6] reported that cellulose and other polysaccharides could be methylated in one step by sodium methylsulfinylmethanide and methyl iodide in a tetramethyl-

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urea–methyl sulfoxide system, but the reported i.r. spectra indicated tetramethylurea to be linked chemically and/or physically to the methylated polysaccharides. Recently, Lomax *et al.*[7] dry-milled cell walls in liquid nitrogen prior to applying the Hakomori method, but severe depolymerisation occurred.

Cellulose can be completely benzylated and allylated in one step by using powdered sodium hydroxide and the corresponding chlorides in the SO_2–diethylamine–methyl sulfoxide system[8,9]. The use of powdered sodium hydroxide has been reported[10] to be effective for methylation of polysaccharides with methyl sulfate in methyl sulfoxide, but was not effective when applied to cellulose[9].

We now report a new, facile methylation method involving powdered sodium hydroxide and methyl iodide, and its application to cellulose powder, wood cellulose, unbleached kraft pulp, wood holocelluloses (cell-wall polysaccharides obtained after delignification of wood meals) with various lignin contents, and cell-wall polysaccharides isolated from the midrib of *Nicotiana tabacum*.

RESULTS AND DISCUSSION

Methylation of cellulosic materials. — Since commercial cellulose powder, wood cellulose, and unbleached kraft pulp prepared from softwoods (N-UKP) are insoluble in methyl sulfoxide, their methylated products have not been obtained even by using the modified[5] Hakomori method.

Many non-aqueous solvents for cellulose are now known[11]. Such solvents have been used for the preparation of various trisubstituted cellulose ethers[8,9] (Table I). Paraformaldehyde and chloral systems are not suitable for the present purpose, because substitution also occurs at the hemiacetal hydroxyl group produced by reaction of cellulose with the aldehydes[12]. Joseleau *et al.*[13] reported the methylation of cellulose in 4-methylmorpholine *N*-oxide using the Hakomori method, but the d.s. of the products was <2.7. Isogai *et al.*[8] found that only with the SO_2–diethylamine–methyl sulfoxide system could tri-*O*-benzylcellulose be prepared quantitatively, and without any depolymerisation, by the use of powdered sodium hydroxide and benzyl chloride under nitrogen, and this system was used

TABLE I

NON-AQUEOUS SOLVENTS FOR CELLULOSE AS METHYLATION MEDIA

Solvent	*Solute*	*Remarks*
Paraformaldehyde–methyl sulfoxide	Cell-O-$(CH_2)_n$-OH[11]	Unsuitable
Chloral–methyl sulfoxide	Cell-O-$CH(CCl_3)$-OH[14]	Unsuitable
4-Methylmorpholine *N*-oxide	Complex[15]	D.s. <2.7[13]
LiCl–*N*,*N*-dimethylacetamide	Complex[16]	Depolymerisation during benzylation[8]
N_2O_4–methyl sulfoxide	Cell-O-NO + HNO_3[11]	Yield <60% for benzylation[8]
SO_2–diethylamine–methyl sulfoxide	Complex[17]	Suitable

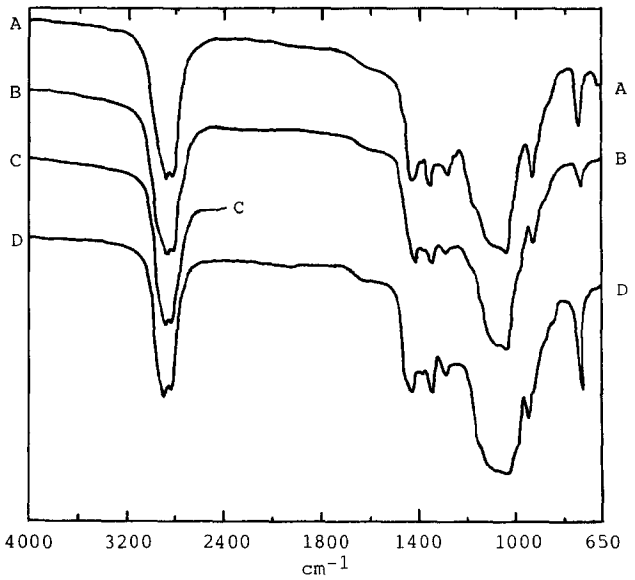

Fig. 1. I.r. spectra of polysaccharides methylated with powdered NaOH and MeI: A, cellulose powder; B, wood cellulose prepared from spruce wood-meals; C, wood cellulose prepared from beech wood-meals; D, N-UKP.

for the methylation of cellulose. Full methylation of cellulose could not be achieved[9] using powdered sodium hydroxide and methyl iodide in the SO_2–diethylamine–methyl sulfoxide system under conditions similar to those used for benzylation with benzyl chloride. However, under milder conditions and as shown by the i.r. spectra in Fig. 1, cellulose powder (A), wood cellulose (B and C), and N-UKP (D) could be completely methylated without the occurrence of side reactions such as oxidation. When these reagents were used with a suspension of cellulose in methyl sulfoxide, the d.s. of the product was <2.6.

Table II shows the compositions and lignin contents of the cellulose samples

TABLE II

SUGAR COMPOSITIONS AND LIGNIN CONTENTS OF CELLULOSE SAMPLES

Sample	*Sugar composition (%)*						*Lignin (%)*
	Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	
Cellulose powder	0	0	0.5	0.5	0	99.0	0
Wood cellulose (Spruce)	0.4	0.5	0.7	3.6	0.7	94.0	0
Wood cellulose (Beech)	0.3	0.5	1.2	0.1	0	97.8	0
N-UKP[a]	0.3	0.5	6.6	6.5	0	81.4	4.7
	(0.3	0.6	6.9	6.8	0	85.4)[b]	

[a]Unbleached kraft pulp prepared from softwoods. [b]Total sugar % is adjusted to 100%.

TABLE III

METHYLATION ANALYSES OF CELLULOSE SAMPLES[a]

Methylated sugar	*Retention coefficient*[b]	*Cellulose powder*	*Wood cellulose (Spruce)*	*Wood cellulose (Beech)*	*N-UKP*[c]
2,3,5-Ara[d]	0.74	0	0	0	0.5
2-Ara	1.02	0	0	0	0.3
(Total Ara)		(0)	(0)	(0)	(0.8)
2,3-, 3,4-Xyl	0 92	0 5	0.6	1.0	4.3
3-Xyl	1.04	0	0	0	1 3
(Total Xyl)		(0.5)	(0.6)	(1.0)	(5.6)
2,3,6-Man	1.11[e]	0	3.5	0	6.0
2,3-Man	1.31	0.5	0.3	0.2	0.7
(Total Man)		(0.5)	(3.8)	(0.2)	(6.7)
2,3,4,6-Gal	1.00	0	0.3	0.2	0
2,3,6-Gal	1.11[e]	0	0.7	0	0.2
(Total Gal)		(0)	(1.0)	(0.2)	(0.2)
2,3,4,6-Glc	0.97	trace	0	0	0
2,3,6-Glc	1 13	97.6	91.7	95.7	83.0
2,6-Glc	1.23	0.4	0.6	0.5	1.1
3,6-Glc	1 26	0.4	0.4	0.5	0.4
2,3-Glc	1.33	0.6	1.9	1.8	2.2
6-Glc	1.36	0	0	0	0
(Total Glc)		(99.0)	(94.6)	(98.6)	(86.7)
D.s. for Glc[f]		2.99	2.97	2.97	2.96

[a]Analysed as alditol acetates by g.l.c. on OV-101 (See Experimental). [b]Relative to that of 2,3,4,6-Gal. [c]Unbleached kraft pulp prepared from softwoods. [d]2,3,5-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [e]As 2,3,6-Man and 2,3,6-Gal have the same retention times, their ratios were measured by g.l.c. on SP-1000; the retention coefficients were 1.30 and 1.34, respectively. [f]Calculated according to the formula,

$$\frac{2,3,6\text{-Glc} \times 3 + (2,6\text{-Glc} + 3,6\text{-Glc} + 2,3\text{-Glc}) \times 2}{2,3,6\text{-Glc} + 2,6\text{-Glc} + 3,6\text{-Glc} + 2,3\text{-Glc}}$$

used, and Table III shows the results of methylation analyses. Each of the cellulose samples contained some sugars derived from hemicelluloses. The sugar compositions determined by methylation analyses (Table III, in parentheses) coincide with those in Table II. Furthermore, the d.s. for the glucose residues in the methylated cellulose was ~3 in accord with the i.r. spectra, and those in methylated wood celluloses and N-UKP were also high. Although it is not clear whether the trace amounts of the di-*O*-methylglucitol derivatives were derived from cellulose or the glucomannan of hemicellulose, their detection indicates the possible presence of glucosyl residues linked at positions 2, 3, and 6 to other sugars. The reactivities of

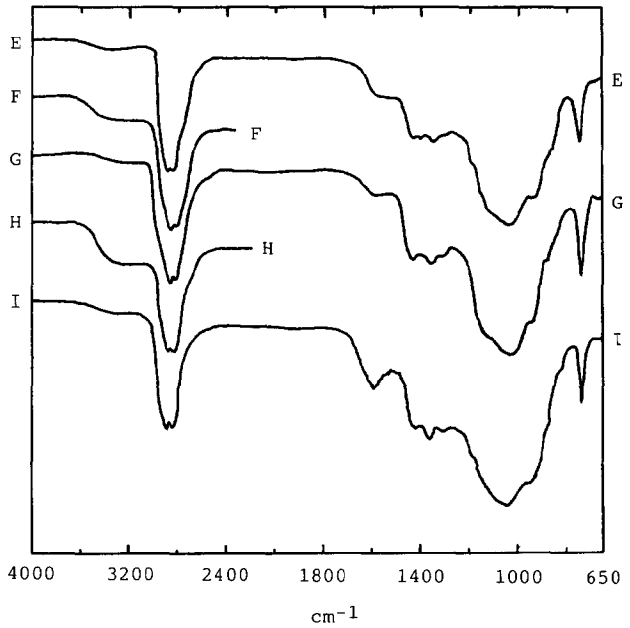

Fig. 2. I.r. spectra of polysaccharides methylated with powdered NaOH and MeI as suspensions in SO_2–diethylamine–methyl sulfoxide: E, spruce-4 (lignin, 2.0%); F, spruce-3 (lignin, 7.3%); G, beech-3 (lignin, 0.2%); H, beech-2 (lignin, 7.9%); I, cell-wall polysaccharides isolated from the midrib of *Nicotiana tabacum*.

TABLE IV

COMPOSITIONS AND LIGNIN CONTENTS OF HOLOCELLULOSES AND CWM[a]

Sample	*Neutral sugar composition (%)*[b]						*Uronic acid (%)*	*Lignin content (%)*
	Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*		
Spruce-4[c]	0.3	1.4	8.5	15.0	1.4	73.9	—[d]	2.0
Spruce-3	0.3	1.5	8.6	16.5	1.2	71.9	—	7.3
Beech-3	0.2	0.8	25.7	1.8	0.5	70.9	—	0.2
Beech-2	0.2	0.6	25.3	1.5	0.5	71.9	—	7.9
CWM[a]	2.2	4.0	13.5	4.1	4.7	71.5	32.8	4.5

[a]Cell-wall polysaccharides isolated from the midrib of *Nicotiana tabacum*. [b]Total % of neutral sugar is adjusted to 100%. [c]See Experimental. [d]Not determined.

HO-2,3,6 toward powdered sodium hydroxide and methyl iodide in SO_2–diethylamine–methyl sulfoxide are similar[18]. Therefore, the relatively high ratios of 2,3-Glc for two wood celluloses shown in Table III suggest the presence of 6-substituted glucosyl residues.

Treatment of polysaccharides with sodium methylsulfinylmethanide, even for 0.5 h under nitrogen, causes some depolymerisation. Thus, the depolymerisation

of cellulose must be significant during the treatment with sodium methylsulfinylmethanide for 12 h (the modified Hakomori method[5]). The formation of trace amounts of 2,3,4,6-Glc, as shown in Table III, indicates that no significant depolymerisation occurred during this new method of methylation, as was observed for benzylation[8].

Methylation of holocelluloses and acidic polymers. — Wood holocelluloses are insoluble in methyl sulfoxide and are rarely fully methylated in one step by the Hakomori method. Even holocelluloses containing <2% of lignin were insoluble and only swelled in the SO_2–diethylamine–methyl sulfoxide system, whereas wood celluloses, prepared by alkali-treatment of the holocelluloses and N-UKP containing 4.7% of lignin, were soluble in this system; wood hemicellulose was not soluble in the system. The insolubility of wood holocelluloses containing ~30% of hemicellulose may arise because the hemicellulose matrix covers the surfaces of the cellulose fibrils, whereas N-UKP containing ~15% of hemicellulose is soluble because of the relatively low content of hemicellulose.

The i.r. spectra in Fig. 2 show that spruce holocelluloses (lignin content, 2.0%; E) and beech holocellulose (lignin content, 0.2%; G) were almost completely methylated in one step, but the spectra of methylated spruce (lignin content, 7.3%; F) and beech (lignin content, 7.9%; H) holocelluloses showed absorption for hydroxyl groups.

As shown in Table IV, cell-wall polysaccharides (CWM) isolated from the midrib of *Nicotiana tabacum* contained ~33% of acidic sugars, and swelled in the SO_2–diethylamine–methyl sulfoxide system. The weak absorption for COONa at ~1600 cm^{-1} in the i.r. spectra of the methylated products (Fig. 2, I) indicates loss of carboxylic groups during methylation. This loss also occurred when the Hakomori method was used[19].

Table IV shows the compositions and lignin contents of wood holocelluloses and CWM, and Table V shows the results of the methylation analyses. The compositions determined by methylation analyses (Table V, in parentheses) coincide well with those in Table IV. Since cellulose is difficult to methylate fully by the usual method, the d.s. for Glc in Table V is the criterion for the extent of methylation. For spruce-4 and beech-3, which have low lignin contents, a high d.s. (~2.9) for the glucose residues was obtained, but for spruce-3 and beech-2 (~7% of lignin) the extents of methylation were much lower. Clearly, the presence of lignin prevents methylation of wood carbohydrates, and only samples containing <5% of lignin are methylated to high extents.

The d.s. of the glucose residues in CWM methylated by our method was almost equal to that obtained with the modified Hakomori method[5], as shown in Table V. The presence of 0.4% of 2,3,4,6-Glc, due to the non-reducing, terminal glucosyl groups in CWM-H, however, suggests that some depolymerisation of glucans occurs on treatment with sodium methylsulfinylmethanide for 12 h. On the other hand, 2,3,4,6-Glc could not be detected in CWM methylated by our method.

The partially methylated Rha, Ara, Xyl, Man, and Gal are derived from

TABLE V

METHYLATION ANALYSES[a] OF HOLOCELLULOSES AND CWM

Methylated sugar	*Retention coefficient*	*Spruce-4*	*Spruce-3*	*Beech-3*	*Beech-2*	*CWM[b]*	*CWM-H[c]*
3-Rha	1.05	0	0	0	0	0.2	0.4
(Total Rha)		(0)	(0)	(0)	(0)	(0.2)	(0.4)
2,3,5-Ara	0.74	0	0	0.1	0	0.9	1.1
2,5-, 3,5-Ara	0.86	0	0	0	0	0	0.3
2,3-Ara	0.91	0.2	0.3	0.1	0	0.5	0.7
2-Ara	1.02	0.5	0.3	0.5	0.5	0.5	0.7
(Total Ara)		(0.7)	(0.6)	(0.7)	(0.5)	(1.9)	(2.8)
2,3,4-Xyl	0.79	0.3	0	0.3	0	1.9	1.8
2,3-, 3,4-Xyl	0.92	7.2	6.1	20.8	20.9	8.4	10.0
3-Xyl	1.04	1.1	2.0	2.0	2.6	1.9	1.8
Xyl	1.15	0	2.0	0.9	2.5	2.0	3.0
(Total Xyl)		(8.6)	(8.3)	(24.0)	(26.0)	(14.2)	(16.6)
2,3,6-Man	1.11	13.2	15.5	1.5	1.3	2.5	5.0
4,6-Man	1.24	0	0	0.1	0	0	0
2,3-Man	1.31	1.1	1.4	0.4	0.3	1.7	0.4
Man	1.64	0	0	0	0.3	0	0.1
(Total Man)		(14.3)	(16.6)	(2.0)	(1.9)	(4.2)	(5.5)
2,3,4,6-Gal	1.00	0.3	0.2	0.1	0	1.0	0.2
2,3,6-Gal	1.11	1.5	1.0	0.5	0.3	2.5	1.9
2,3,4-Gal	1.21	0.1	0	0	0	0.2	0.1
(Total Gal)		(1.9)	(1.2)	(0.6)	(0.3)	(3.7)	(2.2)
2,3,4,6-Glc	0.97	0.1	0	0.1	0	0	0.4
2,3,6-Glc	1.13	69.4	61.0	66.9	48.4	64.8	62.6
2,6-Glc	1.23	1.0	1.6	1.0	1.6	1.3	1.2
3,6-Glc	1.26	0.5	1.0	0.5	2.5	0.6	1.2
2,3-Glc	1.33	1.9	2.8	2.1	4.2	5.0	5.4
6-Glc	1.36	0.4	1.1	0.3	1.8	0.3	0.8
2-Glc	1.48	0	1.3	0.1	2.5	0.2	0.1
3-Glc	1.52	0	0.2	0	0.9	0	0.5
Glc	1.66	1.2	4.0	1.7	9.4	1.6	0.5
(Total Glc)		(74.5)	(73.0)	(72.7)	(71.3)	(73.8)	(72.5)
D.s. for Glc		2.90	2.69	2.87	2.34	2.83	2.84

[a]See Footnote in Table III. [b]Methylated by our method. [c]Methylated by the modified Hakomori method[5].

hemicelluloses. For N-UKP (Table III), spruce-4, beech-3, and CWM, the proportion of 2,6-Glc was about twice that of 3,6-Glc, and the proportion of 2,3-Glc, was about four times that of 3,6-Glc. These results suggest the presence of very small amounts of 1,3,4- and 1,4,6-linked glucosyl residues in each of the four samples. 1,4,6-Linked glucosyl residues are present in xyloglucans, but polysaccharides con-

taining 1,3,4-linked glucosyl residues have not been reported hitherto. Further investigation is required to confirm whether these residues are present in cell-wall polysaccharides or are due to demethylation during hydrolysis of methylated polysaccharides. The relatively high proportions of unmethylated glucose residues probably reflect incomplete methylation.

Thus, the methylation procedure involving powdered sodium hydroxide, methyl iodide, and the SO_2–diethylamine–methyl sulfoxide solvent system for cellulose, holocellulose, and CWM, effected a high d.s. and caused little depolymerisation. As with the Hakomori method, decomposition of uronic acid residues could not be avoided.

EXPERIMENTAL

General. — I.r. spectra were recorded with a Shimazu Model IR-400 spectrometer for films deposited from chloroform solutions or for KBr discs. Commercial cellulose powder (Avicel) and unbleached kraft pulp (from softwoods; Tokai Pulp Co., Ltd.) were used. Holocelluloses with various contents of lignin were prepared from spruce and beech wood-meals (<80 mesh, after extraction with alcohol–benzene) by delignification[20] with sodium chlorite–acetic acid at 75°. Spruce-4 (Tables IV and V), for example, means the spruce-holocellulose prepared from spruce wood-meal by four repetitions of the delignification treatment. Lignin contents were measured by the Klason method[21]. Wood celluloses of spruce and beech were prepared from spruce-4 and beech-3, respectively, by treatment with aqueous 17.5% NaOH containing 4% of H_3BO_3 and 2% of $NaBH_4$ at room temperature under N_2 for 12 h. The alkali-soluble portion (wood hemicellulose) was removed by filtration (glass filter, 1G2), and the residues (wood cellulose) were washed successively with dilute aqueous NaOH, water, dilute acetic acid, water, and acetone. Cell-wall polysaccharides (CWM) were isolated[22] from the midrib of *Nicotiana tabacum*.

Analytical grade methyl sulfoxide was dried over molecular sieve 3A. Methyl iodide was used after distillation. NaOH flakes were technical grade (>96%).

Preparation of polysaccharide solutions or suspensions. — Conc. SO_2–methyl sulfoxide solution (~0.3 g/mL) was prepared as follows: SO_2 (15 g, dried over $CaCl_2$) was bubbled into methyl sulfoxide (50 mL) (exothermic reaction). The SO_2–methyl sulfoxide solution was stable at room temperature for two months in the dark.

Dry cellulose powder, wood cellulose, or N-UKP (1 g) was dispersed in methyl sulfoxide (87.7 mL), the suspension was heated at 60° for 0.5 h and then cooled to room temperature, and the SO_2–methyl sulfoxide solution (containing 1.19 g of SO_2) and diethylamine (1.91 mL) were added successively. The cellulose powder gave a clear solution within 10 min under stirring. However, stirring for ~3 h was required for complete dissolution of wood celluloses and N-UKP.

The above conditions were used to prepare suspensions of holocelluloses or

CWM, which were stirred at room temperature for 1 h to swell the samples sufficiently.

Methylation of polysaccharides. — To a solution or suspension containing 1 g of polysaccharide was added freshly powdered NaOH (12 g) under N_2 at room temperature, and the mixture was stirred for 1 h under N_2. Methyl iodide (~12 mL) was then added dropwise at room temperature, and the mixture was stirred for 1 h, and then kept at 40° for 0.5 h, at 50° for 0.5 h, and at 60° for 1 h. If solidification occurred during stirring, the minimal amount of methyl sulfoxide to regenerate the slurry was added. The methylated products were isolated by dialysis and subsequent lyophilisation, and hydrolysed[23] first with aqueous 90% formic acid and then 0.125M sulfuric acid. The hydrolysates were neutralised with barium carbonate and centrifuged, and the partially methylated sugars were reduced with $NaBH_4$ and then acetylated[24]. The partially methylated alditol acetates were subjected to g.l.c. using a capillary column (50 m × 0.27 mm) coated with silicon OV-101, a temperature programme 150°→220° at 2°/min, and a helium flow-rate of 1 mL/min. For separation of 2,3,6-Man and 2,3,6-Gal, a capillary column (40 m × 0.28 mm) coated with SP-1000 was used. Peak areas were measured with a Hewlett–Packard 3380A integrator. G.l.c.–m.s. was performed with a Hitachi M-80 mass spectrometer (20 eV), using a column of OV-101. The component in each peak was identified by its fragmentation pattern and retention time[25].

For the determination of sugar compositions, samples were hydrolysed by the method of Saeman *et al.*[26]. After treatment with aqueous 72% sulfuric acid for 4 h at 20°, the mixture was diluted with water to 3% sulfuric acid, stored for 1 h at 120°, and neutralised with barium carbonate, and the monosaccharides were analysed as alditol acetates by g.l.c. using a column (2 m × 3 mm) containing Gas Chrom P coated with a mixture[24] of 0.2% PEGA, 0.2% PEGS and 0.4% silicone XF-1150.

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