Reduction of the aldehyde groups with sodium [^{3}H]tetrahydroborate (0.6 GBq/mmole) led to the formation of (II) with a specific activity of 1.2-1.4 GBq/g, i.e., corresponding to 0.2% of modified groups of the cellulose. The reaction was carried out in physiological solution for an hour, and then a 1% solution of unlabeled sodium tetrahydroborate was added. Compound (III) was obtained by the interaction of activated cellulose with [2-^{14}C]lysine (1800 MBq/mmole) followed by reduction with sodium tetrahydroborate to stabilize the bond formed between the amino acid and the polysaccharide and to reduce residual aldehyde groups. The specific activity of compound (III) was 120 MBq/g, which corresponds to 1% of modified groups of the cellulose.

To obtain (IV), the oxidized cellulose was coupled with hexamethylenediamine, and then the aminohexylcellulose formed was treated with [1-^{14}C]acetaldehyde (200 MBq/mmole) in 0.01 M sodium bicarbonate buffer and was reduced with sodium tetrahydroborate. The specific activity of (IV) was 100-120 MBq/g, and the percentage of modified groups in the cellulose was 4.0.

The (IV) preparation obtained was injected into mice of the line (CBA × C57B/6)G with a total radioactivity of 0.185 MBq per animal. It was found that 6.3% of the label in the dose administered was excreted in the course of two weeks. In an investigation of the tissues of the animals it was found that the radioactivity was localized mainly at the site of injection of the preparation and in the liver and the spleen. The results obtained show that in the excretion of cellulose the main role is played by the RES.

Thus, a series of radioactive forms of cellulose has been obtained which provide the possibility of investigating the mechanism of the cooperative response of immune cells to the introduction of a natural polymer into the organism.

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SELECTIVE BINARY MOBILE PHASE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF 12,13-EPOXYTRICHOTHEC-9-EN-8-ONES

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In the present paper we consider questions of the optimization of the composition of the mobile phase (MP) for HPLC of the group of 12,13-epoxytrichothec-9-en-8-ones (I-V) formed by the fungus Fusarium graminearum Schw., which are contaminants of fusarial grain [1, 2]. It is known that a MP containing 30% of methanol in water ensures the separation of trichothecenes (I), (III), and (IV) [3], but the separation of (II) and (III) is achieved only at methanol concentrations of 7 and 10% [4, 5], which makes the combined determination of the whole group of substances impossible because of the extreme increase in the capacity coefficients for trichothecenes (IV) and (V).

The chromatographic analysis of the mixture consisting of nivalenol (I), 4,7-dideoxynivalenol (II), 4-deoxynivalenol (III), 15-acetyl-4-deoxynivalenol (IV), and 3-acetyl-4-deoxynivalenol (V) was carried out on a Milikhrom chromatograph fitted with a 2 × 62 mm microcolumn filled with the sorbent Nucleosil C18 (5 μm) at a rate of flow of eluent of 50 μl/min. The substances were detected UV-spectrophotometrically at a wavelength of 224 nm, and as organic modifiers for the MP we used ethanol, acetonitrile, and tetrahydrofuran (THF).

On the use of a MP containing ethanol in amounts of 15 and 20%, the values of the capacity coefficients K' of the substances being analyzed were in the acceptable interval of 1.4 to 15.2 but trichothecenes (IV)/(V) were not separated satisfactorily (α = 1.05), and (II)/(III) were not separated.

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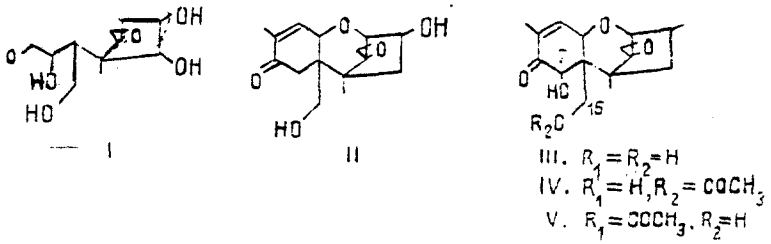
 0009-3130/89/2506-0732$12.50



I II

III. $R_1 = R_2 = H$
IV. $R_1 = H, R_2 = COCH_3$
V. $R_1 = COCH_3, R_2 = H$

In the MP water–acetonitrile containing 33, 40, and 50% of acetonitrile, separation of all the components was achieved but with low selectivity for the substance pairs (II)/(III) and (IV)/(V) (Table 1). The capacity coefficients of the substances being analyzed were between 1.06 and 17.0.

At a proportion of 33% of acetonitrile in the MP, the trichothecines were eluted in the sequence (I) < (III) < (II) < (V) < (IV). At 40 and 50% of acetonitrile, the monoacetates (IV) and (V) were eluted in the reverse sequence – 15-acetyl-4-deoxynivalenol (IV) was eluted earlier than the 3-acetate (V). This change in the elution sequence is apparently connected with the greater interaction of the 15-acetate with the MP on a decrease in its polarity in comparison with the 3-acetate because of the existence in the molecules of 15-acetate (IV) of an intramolecular bond of the OH group at C_7 with the carbonyl group of the acetyl radical at C_{15}.

This selectivity of the separation of the substance pairs (II)/(III) was practically constant (α = 1.08-1.09) over the whole interval of concentrations of acetonitrile used, while the highest selectivity (α = 1.11 for the pair of monoacetates (IV)/(V)) was observed at 33% of acetonitrile, when their retention and, consequently, the diffuseness of the peaks and the time of analysis were excessively large (K' for (IV) and (V) 17.0 and 15.3, respectively).

The selectivity necessary for the complete separation of all the components of the mixture was achieved in the MP water–THF (Table 2). THF, being a weakly polar modifying agent capable of forming hydrogen bonds, ensures the separation of the trichothecenes both through the difference in the positions of the acetyl residues and through the difference in the number of hydroxy groups.

The order of elution of the substances (I) < (II) < (III) < (IV) < (V) in the MP water–THF did not change over the whole interval of concentrations of THF that were used. In comparison with the MPs containing 7 and 10% of methanol in water [4, 5] and also 40 and 50% ofacetonitrile in water (see Table 1), the order of emergence of the trichothecenes (II) and (III) changed to the opposite: 4,7-dideoxynivalenol (II) was eluted earlier than 4-deoxynivenol (III). Apparently, the existence in the molecule of 4-deoxynivalenol (III) of an intramolecular bond of the carbonyl group at C_8 with the OH group at C_7 weakens its interaction with the MP when THF is used as the modifying agent.

As was to be expected, being a weakly polar modifying agent, THF had a greater effect on the separation of hydrophobic adsorbates (see Table 2). With an increase in its concentration in the MP the selectivity of the separation of substances (IV)/(V) increased with no change in the selectivity of the separation of the trichothecenes (II)/(III). An increase in the amount of THF in the MP raised the selectivity of separation of the trichothecenes (V)/(IV) and shortened the time of analysis, but at the same time it decreased the retention of trichothecenes (I) and (II) excessively. The best separation of all the components and

TABLE 1. Dependence of the Parameters of HPLC for Trichlothecenes (I-V) on the Composition of Water–Acetonitrile MPs

Proportion of acetonitrile, vol. %	Retention volume, ml						α, selectivity	
	I	III	II	V	IV	III/I	II/III	IV/V
33	214	341	364	1450	1600	2,02	1,09	1,11
36	210	303	321	1310	1310	1,77	1,08	1,00
	I	III	II	IV	V	III/I	II/III	V/IV
40	200	260	274	878	925	1,53	1,08	1,06
50	183	221	233	539	580	1,40	1,09	1,08

TABLE 2. Dependence of the Parameters of the HPLC Separation of the Trichothecenes (I-V) on the Composition of a Water–THF MP

Proportion of THF, vol. %	Retention volume, μl					α, selectivity		
	I	II	III	IV	V	II/I	III/II	V/IV
15	234	313	356	1257	1296	1,55	1,19	1,02
18	205	254	287	756	811	1,42	1,20	1,03
20	201	250	281	727	782	1,43	1,19	1,09
25	185	226	256	567	628	1,42	1,18	1,13
30	179	216	240	432	491	1,41	1,19	1,17

an acceptable time of analysis on a microcolumn of 14 min, was achieved in a MP containing 25% of THF.

The separation of 3-acetyl- and 15-acetyl-substituted 4-deoxynivalenols under the conditions of reversed-phase HPLC has not been reported previously. This is the fist time that the use of MP water–THF in the HPLC of the trichothecenes has been described.

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