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PRODUCTION OF SORBENTS FOR REVERSED-PHASE CHROMATOGRAPHY BY THE ALKYLATION OF THE SURFACE OF SILICA GEL WITH ALCOHOLS

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A procedure is proposed for alkylating the surface of silica gel with heptyl, decyl, and dodecyl alcohols with the aim of obtaining sorbents for reversed-phase chromatography. The efficacy of the use of the sorbents obtained for the purification and separation of triterpene glycosides has been shown.

The chemical modification of the surface of silica gels by various organic and inorganic substances permits the purposeful production of sorbents with new properties [1]. In liquid reversed-phase chromatography, sorbents with grafted-on alkyl groups (1-22 methylene units) are widely used, while more than half the sorbents marketed abroad have been modified by treating the surface of silica gel with octadecyldimethylchlorosilane – the so-called ODS sorbents [2]. They are distinguished by a high hydrolytic stability of the Si–O–Si–C bond of the modifying agent with the support.

Supports with grafted-on alkoxy groups are also fully suitable for preparative liquid chromatography even though the Si–O–C bond is not as resistant to hydrolysis, particularly at elevated temperatures. The alkylation of activated silica gel with (C_1-C_8) alcohols was first described by Deuel et al. [3]. However, the degrees of coverage that they achieved were low in comparison with modern ODS sorbents, and the chromatographic characteristics of the products were not described.

We propose a procedure for alkylating the surface of silica gel with alcohols in which a high degree of coverage of the surface by alkoxy groups and good chromatographic characteristics in the separation of triterpene glycosides are achieved.

The surface of silica gel after hydroxylation and drying in vacuum with the aim of eliminating physically adsorbed water was activated by converting the surface silanol Si–OH groups into Si–Cl groups by treatment with thionyl chloride in benzene. Then the chlorinated silica gel was treated with the modifying alcohol in the presence of pyridine in boiling benzene.

Table 1 gives the results of the analysis of silica gel L (Czechoslovakia) alkylated with heptyl, decyl, and dodecyl alcohols. The percentages of alkyl groups calculated on the basis of the results of microanalysis for carbon and of thermogravimetric results practically coincided. The densities of grafting calculated in the light of the specific surface of silica gel L, 600 m^2/g [2], were comparable with results of investigations in which alkyldimethylchlorosilanes were used as modifying agents [4, 5].

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TABLE 1. Characteristics of the Modified Silica Gels

Grafted group	C found, %	Calculated C_nH_{2n+1}, %	Found Δm, %	Density of grafting		
				mmole/g	μmole/m²	groups/nm²
C_7H_{15}	10,9	12,8	14,5	1,29	2,15	1,29
$C_{10}H_{21}$	14,2	16,7	18,0	1,18	1,97	1,18
$C_{12}H_{25}$	15,0	17,6	19,0	1,04	1,73	1,04

In order to study the chromatographic characteristics of the adsorbents obtained, we carried out the separation of pairs of triterpene glycosides differing by the structure of the carbohydrate moiety. A fairly good separation was achieved of 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oleanolic acid and 3-O-[O-β-D-glucopyranosyl-(1 → 2)-O-β-D-glucopyranosyl]hederagenin [6], which are difficult to separate on unmodified silica gel L. On the other hand, the separation of the pairs 3-O-α-L-arabinopyranosyl-, 28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin and 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-, 28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-Oβ-D-glucopyranosyl-(1 → 6)-β-D-glucopyanosyl]hederagenin [7] was successfully carried out both on the unmodified and on the modified silica gel; however, in the latter case the time of separation was considerably shortened. Moreover, in the case of chromatography on the modified silica gel the triterpene glycosides isolated were free from flavonoids and other phenolic compounds, since they had very short retention times on the adsorbent and were eluted first. Water–methanol and water–ethanol solvent systems proved to be suitable for elution. However, the best separation and a substantially higher rate of elution were achieved on the use of water–acetonitrile systems. The greatest efficacy of the separation of the pairs of glycosides used was achieved on silica gel with dodecyl groups.

EXPERIMENTAL

Thermogravimetric analysis was performed with a Q-derivatograph on the Paulik–Paulik–Erdey system (Hungary) in a static air atmosphere; the rate of heating was 10°C/min, the weight of the sample about 0.500 g, the sample holder was a platinum crucible without a lid, and the standard was calcined Al_2O_3. A loss in mass was observed on the heating curves in the temperature interval of 200-700°C; in the differential thermogravimetric curves an extremum in the 230°C region corresponded to an exothermal effect.

TLC analysis was carried out on Silufol plates (Czechoslovakia). The spots of the glycosides were detected by spraying the chromatograms with 10% perchloric acid followed by heating to 110-120°C. For TLC we used the solvent systems chloroform–methanol–ammonia (7:3:1), and for column chromatography the systems were 1) water–ethanol (6:4); 2) water–ethanol (5:5); 3) water–methanol (6:4); 4) water–methanol (5:5); 5) water–acetonitrile (7:3); and 6) water–acetonitrile (6:4).

Hydroxylation of the Silica Gel. Silica gel L 100-160 μm (Czechoslovakia) was hydroxylated by boiling in distilled water for 24 h and was filtered off and dried in vacuum (10 mm Hg) at 110-120°C to constant weight.

Chlorination of the Silica Gel. With stirring, 100 g of the hydroxylated silica gel in 300 ml of dry benzene was treated with 22 ml (0.3 mole) of thionyl chloride, which was added in portions over 1 h with subsequent heating for an hour at the boiling point of the benzene until the evolution of gaseous hydrogen chloride ceased. After cooling, the benzene solution was decanted off from the deposit of chlorinated silica gel.

Alkylation of the Silica Gel. With stirring, 300 ml of dry benzene, 57 ml (0.7 mole) of pyridine and, in portions over an hour, 0.3 mole of an alcohol (42 ml of heptan-1-ol or 56 ml of decan-1-ol, or 68 ml of dodecan-1-ol) were added to a reaction flask containing the chlorinated silica gel, and stirring was continued at the boiling point of the solvent for 5 h. After cooling, the deposit of alkylated silica gel was filtered off with suction in a funnel with a glass filter and was washed successively with benzene, chloroform, ethanol, and water. The product obtained was dried in the air and, for analysis, additionally in vacuum (1 mm Hg) at 80°C to eliminate the adsorbed water completely.

Evaluation of Chromatographic Properties. For separating the glycosides on the alkylated silica gels we used a column with a D/H ratio for the adsorbent in it of 1:20, the ratio of adsorbent to adsorbate being 1:1000. We separated mixtures of the triterpene gly-

cosides 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oleanolic acid and 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]hederagenin [6] with elution by solvent systems 2, 4, and 6, and also mixtures of 3-O-α-L-arabinosyl-28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 5)-β-D-glucopyranosyl]hederagenin and 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-28-O-[O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl]hederagenin with elution by solvent systems 1, 3, and 5. Samples of the eluates were analyzed by TLC. The chromatographic characteristics of the alkylated silica gels and the amounts of modifying agents remained stable over a year.

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ALKALOIDS OF Aconitum coreanum.

III. 13-ACETYL-14-HYDROXY-2-ISOBUTYRYLHETISINE N-OXIDE

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It has been established by the x-ray structural method that a new alkaloid isolated from the epigeal part of Aconitum coreanum has the structure of 13-acetyl-14-hydroxy-2-isobutyrylhetisine N-oxide. This is the first time that a N-oxide has been isolated among the hetisine alkaloids.

The isolation from the epigeal part of Aconitum coreanum (Levl) Rapaics of four alkaloids belonging to the hetisine and atisine types has been reported previously [1, 2]. Continuing investigation of the alkaloids of this plant from the mother liquors before the isolation of 14-hydroxy-2-isobutyrylhetisine (Guan-Fu base Z) we obtained a crystalline mixture of perchlorates which, by the usual treatment, was converted into a mixture of bases. The column chromatography of this mixture gave a new base with mp 240-242°C, M^+ 473, which proved to be 13-acetyl-14-hydroxy-2-isobutyrylhetisine N-oxide. Its perchlorate had mp 285-288°C (from ethanol). The IR spectrum of the perchlorate showed the absorption bands of hydroxy groups (3500, 3400 cm^{-1}), of an ester carbonyl (1740, 1720 cm^{-1}), and of a double bond (1660, 890 cm^{-1}). According to its PMR spectrum, the base contained an exomethylene group (broadened singlet at 4.90 and 4.99 ppm), one acetoxy group (singlet, 3H at 2.04 ppm), an isobutyryloxy group (two doublets, 3 H each, at 1.24 and 1.15 ppm, J = 4 Hz) and a methyl group (singlet, 3 H at 1.20 ppm).

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