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USE OF N-PROTECTED AMINOALKOXYAMINES IN SYNTHESIS OF AMINOOXY ADSORBENTS USING BrCN

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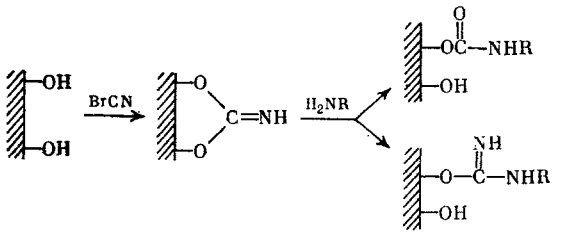
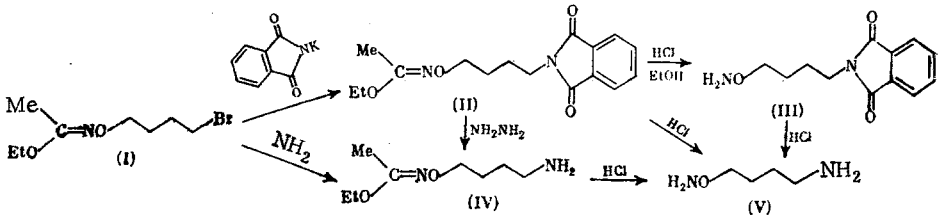
Up to now aminooxy adsorbents, exemplified by the polymeric O-protected hydroxylamines, have been synthesized by the alkylation of cellulose [1] and dextrans [2] using haloalkylhydroxylamines; here the O-protected hydroxylamine moiety was attached to the matrix by a stable ether linkage. Unfortunately, a number of polysaccharides, which proved to be unstable under the previously employed synthesis conditions (using liquid NH_3 as the solvent), could not be converted to aminooxy adsorbents in this manner. A variation in the synthesis of aminooxy adsorbents, which makes it possible to insert an O-protected hydroxylamine moiety into an agarose gel, is discussed in the present communication.

A widely used method for the modification of polysaccharides is the preparation of their iminocarbonate derivatives by treatment with either BrCN [3] or isocyanates [4], followed by coupling with various nitrogen bases [5].



In this connection we studied the reaction of BrCN-activated Sepharose with ethoxyethylideneaminooxybutylamine, the synthesis of which, together with that of some related compounds, is depicted in the following scheme:



The alkylation of potassium phthalimide with bromide (I) leads to the (II) derivative, the mild hydrolysis of which gave hydrochloride (III), while hydrazinolysis gave the desired product (IV). Products (II), (III), and (IV) when treated with excess HCl are converted to

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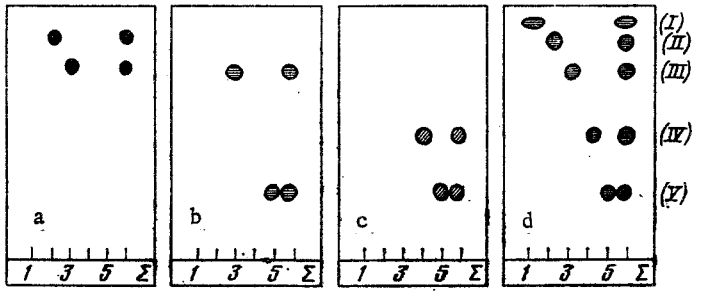

Fig. 1. Chromatogram of (I)-(V) (isopropanol—NH_4OH—H_2O = 7 : 1 : 2): a) in UV (λ_{excit} 254 nm); b) after treatment with pyridoxal 5'-phosphate solution, in UV (λ_{excit} 360 nm); c) the same, in HCl vapors; d) the same, after transfer to an NH_3 atmosphere, in UV (λ_{excit} 360 nm).

dihydrochloride (V). The direct alkylation of NH_3 with bromide (I) also led to amine (IV), in which connection the yield of (V) invariably proved to be higher by the route (I) → (II) → (V), whereas the yield of (IV) was higher in the direct reaction, which is apparently associated with the sensitivity of the ethoxyethylidene protective group toward hydrazine.

The structure of all of the synthesized derivatives was confirmed by elemental and functional analysis. In running the latter we employed our previously developed [6] chromatographic method using pyridoxal 5'-phosphate. Derivatives (I)-(V) were chromatographed on Silufol UV-254 plates. Phthalimido derivatives (II) and (III) were detected using UV absorption. Alkoxyamines (III) and (V), which form fluorescent oximes, were developed after spraying the chromatogram with pyridoxal 5'-phosphate solution. Subsequent transfer of the chromatogram to an HCl atmosphere developed amines (IV) and (V) as bright yellow spots on a white background. Simultaneous hydrolysis of the ethoxyethylidene protective group occurred here: transfer of the chromatogram to an NH_3 atmosphere restored the fluorescence of the pyridoxal 5'-phosphate oximes, in which connection the newly developed spots corresponded to the ethoxyethylidene derivatives (I), (II) and (IV) (Fig. 1).

The synthesized amine (IV) was reacted with BrCN-activated Sepharose [Eq. (1), R = $(CH_2)_4$-$ONC(CH_3)OC_2H_5$]. Aqueous DMF was used as the solvent, which dissolves (IV) and does not destroy the structure of the gel. Removal of the protective group by mile acid hydrolysis led to the aminooxysepharose. Another variation was also tested: the BrCN-activated sepharose was treated with aqueous tetramethylenedihydroxylamine solution [Eq. (1), R = $O(CH_2)_4ONH_2$].

The modified sepharoses, obtained by these procedures, were completely stable in nearly neutral aqueous solutions, which are of greatest interest for biochemical studies, and, similar to previously known aminooxy adsorbents, entered into reactions that are characteristic for O-substituted hydroxylamines.

Studies using the obtained aminooxysepharoses are being conducted at the present time and will be the subject of subsequent publications.

EXPERIMENTAL

1-Bromo-4-(ethoxyethylideneaminooxy)butane (I), n_D 1.4630, and tetramethylenedihydroxylamine dihydrochloride (VI), mp 240°C, were obtained as described in [1]. The Sepharose 4B (Pharmacia, Sweden) and pyridoxal 5'-phosphate (Serva, Federated Republic of Germany) were commercial products. The capacity of the aminooxysepharoses was determined as described in [1].

1-(Ethoxyethylideneaminooxy)-4-phthalimidobutane (II). A stirred mixture of 5.0 g (0.027 mole) of potassium phthalimide and 6.0 g (0.025 mole) of (I) in 20 ml of abs. DMF was heated at 70° for 12 h, after which it was cooled, diluted with 20 ml of water, extracted with $CHCl_3$, and the chloroform extracts were washed in succession with 0.5 M NaOH solution and water. After drying, the solvent was distilled off, and the residual oil was recrystallized from abs. ether. Recrystallization from MeOH gave 6.5 g (82.5%) of (II), mp 40°. Found: C 63.18; H 6.96%. $C_{16}H_{20}N_2O_4$. Calculated: C 63.14; H 6.62%.

1-Aminooxy-4-phthalimidobutane Hydrochloride (III). The hydrolysis of (II) with an equimolar amount of conc. HCl in EtOH solution gave (III), mp 173°. Found: C 53.90; H 5.82; Cl 13.07%. $C_{12}H_{13}N_2O_3Cl$. Calculated: C 53.24; H 5.56; Cl 13.09%.

1-(Ethoxyethylideneaminooxy)-4-aminobutane (IV). 1) A solution of 24 g (0.1 mole) of (I) in 250 ml of dry NH_3 was stirred for 72 h, the NH_3 was evaporated, and the residue was distilled over KOH. Redistillation gave 12.5 g (72%) of (IV), bp 67° (2 mm); n_D 1.4380. Found: C 55.59; H 10.04%. $C_8H_{18}N_2O_2$. Calculated: C 55.15; H 10.41%.

2) To 15 g (0.05 mole) of (II) in 100 ml of MeOH was added 0.05 mole of 85% aqueous hydrazine hydrate and the mixture was refluxed for 1 h, evaporated in vacuo, extracted with hexane, and then vacuum-distilled. Redistillation gave 4.5 g (52%) of (IV).

1-Aminooxy-4-aminobutane Dihydrochloride (V). The hydrolysis of (II), (III) or (IV) with conc. HCl gave (V) in 90-95% yield, mp 232°. Found: C 27.25; H 8.19; N 15.74; Cl 39.76%. $C_4H_{14}N_2OCl_2$. Calculated: C 27.13; H 7.97; N 15.82; Cl 40.04%.

Aminooxybutylsepharose. 1) To 50 ml of settled Sepharose 4B suspension, activated with 10 g of BrCN as described in [5], which had been washed in the cold with 250 ml of 0.1 M sodium borate buffer solution, pH 10.0, and then suspended in the same buffer (total volume = 100 ml) was added, with vigorous stirring and cooling in ice, a solution of 2 g (0.0115 mole) of (IV) in 50 ml of DMF. The suspension was stirred for 12 h at 4°, filtered, and then washed on the filter for 30 min with 0.01 M HCl and 0.1 M NaOAc solution (600 ml) until the test for alkoxyamines with pyridoxal 5'-phosphate was negative.

2) To 25 ml of settled Sepharose 4B suspension, activated and washed as described above, with stirring and cooling in ice, was added 1.1 g (0.0057 mole) of tetramethylenedihydroxylamine dihydrochloride in 45 ml of water. The suspension was stirred for 12 h at 4°, and then washed on the filter with 0.1 M NaOAc solution until the removal of alkoxyamines was complete. In both cases the capacity of the obtained aminooxysepharoses toward H_2NO groups was ~0.1.

CONCLUSIONS

1. The selective removal of protective groups was studied for some O-(aminoalkyl)hydroxylamines, and a method for the functional analysis of the obtained compounds was discussed.

2. Starting with a BrCN-activated Sepharose we obtained some sorbents that carry O-substituted hydroxylamine moieties.

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