rostane disulfate were 2.90 and 12, respectively, in chloroform/methanol, 1:1, containing NaCl (*i.e.* larger than with methylated Sephadex). The retention volume of cholesterol was about the same as with methylated Sephadex. These results indicate that the elution volumes are determined by a partition between a stationary gel-solvent phase and a less polar mobile solvent phase.

When water was present in the sample applied to the column considerable changes in the relative elution volumes were noted. Thus, when sodium D—S was dissolved in 8 ml chloroform/methanol/water, 1:1:0.3, containing 0.01 M KCl and applied to an 8 g Sephadex LH-20 column eluted with chloroform/methanol, 1:1, containing 0.01 M KCl, the relative elution volume of the steroid conjugate was 1.12 as compared to 3.7 when no water was present in the sample. Intermediate water contents gave broad double peaks between the relative elution volumes 1.12 and 3.7.

Applications. Chromatography on methylated Sephadex should be a useful technique for the group separation of free and conjugated steroids and bile acids (Table 1). The method has been used to purify steroid sulfates in extracts of human serum.[5,10,11] In this case it is not necessary to add an electrolyte to the solvent (see above). Phospholipids, triglycerides, esterified and free cholesterol, and free steroids are eluted before the steroid monosulfates.[2] Steroid disulfates are eluted in a later fraction.

When the technique is used for the purification of reaction mixtures in the synthesis of steroid sulfates the addition of electrolytes to the solvents is undesirable. However, when the columns were prewashed with 0.01 M NaOH or NaCl in chloroform/methanol, 1:1 (200—300 μequiv./25 g methylated Sephadex) followed by chloroform/methanol, 1:1 (50—100 ml/25 g) the same elution volume for mg amounts of sodium salts of steroid sulfates were obtained whether the solvent contained NaCl or not.

Recovery studies with labeled D—S and various unlabeled steroid monosulfates have shown that losses on columns of methylated Sephadex are negligible.[5,10]

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Crystal Structure of the *trans* Form of 1,4-Aminomethylcyclohexanecarboxylic Acid

P. GROTH

Universitetets kjemiske institutt, Blindern, Oslo 3, Norway

One of the stereo-isomers of 1,4-aminomethylcyclohexanecarboxylic acid exhibits a strong antifibrinolytic activity, the other almost none.[1]

The hydrobromides of both forms of the amino-acid have recently been examined by X-ray crystallographic methods, and it has been established that the "active" form is the *trans* isomer.[2,3]

In order to obtain precise informations regarding the *trans* isomer of the amino-acid itself, a crystal structure analysis has been carried out.

The crystals are orthorhombic with lattice parameters

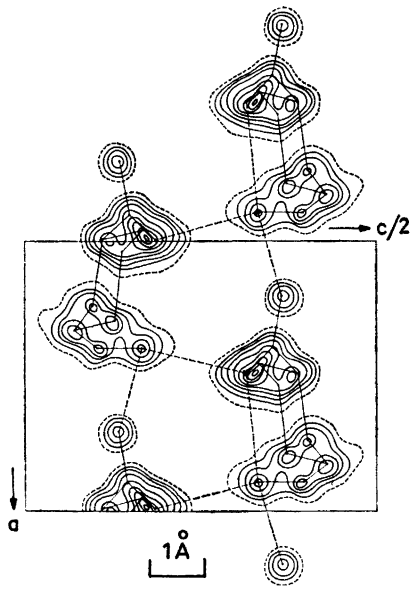

Fig. 1. Fourier projection along *b*-axis. (Dotted circles correspond to atoms in adjacent cells.)

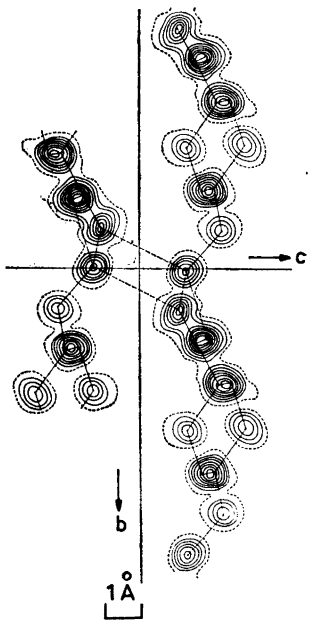

Fig. 2. Fourier projection along *a*-axis. (Dotted circles correspond to atoms in adjacent cells.)

$a = 6.27_9$ Å, $b = 7.89_5$ Å, $c = 16.70_6$ Å

The space group is $P2_12_12_1$ and the cell contains 4 molecules.

Using a computer procedure based on the Cochran-Douglas method [4] (programmed in FORTRAN IV for UNIVAC 1107 by the author) signs of 62 $0kl$-reflections were determined. The corresponding electron density map could easily be interpreted; the R_{0kl}-value arrived at was 12.1 %, and it turned out that 60 signs had been correctly determined.

Using the established z-parameters, approximate x-coordinate values were obtained by allowing the $h0l$-projection of the molecule (determined from a model) to move in small steps parallel to the x-axis and calculating the R-factor for each step. The parameters corresponding to the minimum R-value were chosen as starting coordinates for a full matrix least squares refinement including all $h0l$- and $0kl$-reflections.

The R-value thus arrived at was 8.6 % and the final Fourier maps are reproduced in Figs. 1 and 2. Both maps show considerable overlapping, and publication of interatomic distances and angles will therefore be postponed until the three-dimensional analysis, now in progress, has been finished. The following preliminary results may, however, be stated:

The *trans* form of the amino-acid has the *di*-equatorial conformation, the environment of the nitrogen atom being nearly "tetrahedral" with three independent N—O distances somewhat shorter than 2.80 Å. The C—O—N angles are 130°—140°, one of the oxygen atoms being linked to *one* nitrogen atom, the other to *two* nitrogen atoms. The N—O—N angle corresponding to the latter two bonds is between 90° and 100°.

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