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Note

Studies on steroids

CXLVI. Chromatographic behaviour of organic sulphates on Sephadex LH-20

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The occurrence of numerous kinds of organic sulphates as natural products[1,2], metabolites of drugs and steroid hormones[3] is well documented and a variety of chromatographic methods have been applied to the separation of these highly polar compounds. These methods involve the separation of molecular species of different anionic moieties because they are incapable of differentiating cationic moieties from one another. This paper deals with the chromatographic behaviour of organic sulphate salts consisting of various cations on Sephadex LH-20.

EXPERIMENTAL

Instruments

A Hitachi Model 124 ultraviolet spectrophotometer and a Hitachi Model 208 atomic-absorption spectrophotometer were used.

Reagents and materials

Sephadex LH-20 (particle size 25–100 μm) was supplied by Pharmacia (Uppsala, Sweden). All reagents and chemicals used were of analytical-reagent grade. Distilled water was deionized by percolation through a mixed-bed column of ion exchangers. Dowex 50W-X8 (100–200 mesh) ion-exchange resin was conditioned in the usual manner and then converted into various cationic forms by washing with 2 *N* NaOH, KOH, Li_2SO_4, RbCl, CsCl, $AgNO_3$ and $BaCl_2$ solutions. Marinobufagin 3-sulphate sodium salt was obtained from toad venom in these laboratories. Other salts were prepared by passing an aqueous solution of the sodium salt through a column packed with Dowex 50W-X8 resin in the corresponding cationic form. *p*-Toluenesulphonic acid and benzoic acid were similarly converted into the desired salts as described above.

Chromatography on Sephadex LH-20

Sephadex LH-20 was swollen in each solvent overnight and then packed in a column (35 × 1.5 cm I.D.). The void volume was estimated to be 18 ml when

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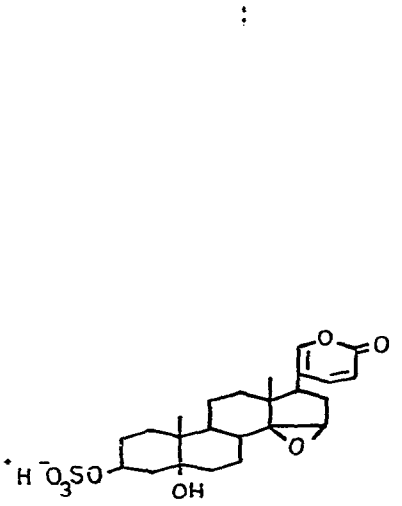
methylated Blue Dextran 2000 (methanol) and Blue Dextran 2000 (water) were used. A synthetic mixture of sulphate salts (each *ca.* 1 mg) dissolved in solvent (1–2 ml) was applied to the column and then eluted with each solvent at a flow-rate of 0.6ml/min.

RESULTS AND DISCUSSION

When submitted to gel chromatography on Sephadex LH-20 using methanol as solvent, a mixture of marinobufagin 3-sulphate (Fig. 1), lithium, sodium and potassium salts were distinctly separated, as illustrated in Fig. 2. A mixture of *p*-toluenesulphonic acid lithium, sodium, potassium and silver salts showed a similar chromatogram under the same conditions. In addition, *p*-toluenesulphonic acid lithium salt and marinobufagin 3-sulphate potassium salt were resolved to give the corresponding peaks (Fig. 3).



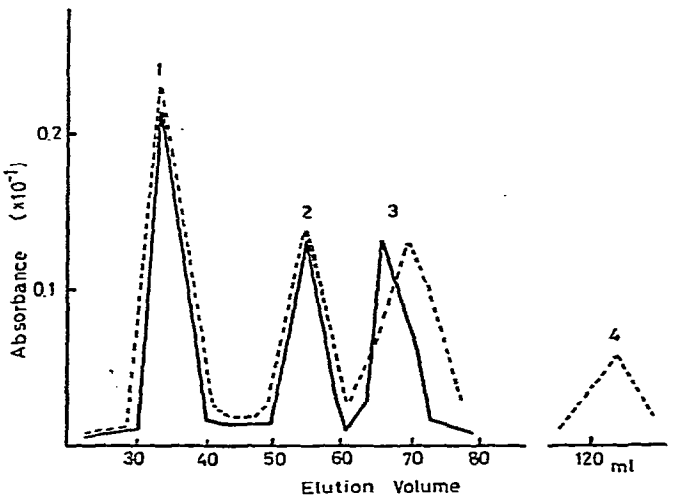


Fig. 1. Structure of marinobufagin 3-sulphate.

Fig. 2. Separation of various salts of marinobufagin 3-sulphate (——) and *p*-toluenesulphonic acid (– – – –) on Sephadex LH-20. Solvent: methanol. Detection: ——, 300 nm; – – – –, 260 nm. 1 = Li, 2 = Na, 3 = K and 4 = Ag salt.

The eluate in each fraction was unequivocally characterized by flame spectrophotometry or the silver chloride test and by converting the cationic moieties into one another by ion-exchange chromatography on Dowex 50W-X8 resin. These experiments revealed that no exchange of cationic moiety occurred among the sulphate salts during gel chromatography and the separation was solely dependent upon the cationic moiety of the sulphate. However, a mixture of benzoic acid lithium, sodium, potassium and silver salts provided no satisfactory resolution except for the lithium salt under the same conditions. Also, *p*-toluenesulphonic acid lithium, sodium and potassium salts were not separated, exhibiting a single peak when water or methanol containing 0.01 *M* sodium chloride was used as solvent. The divalent metal (barium) salts of marinobufagin 3-sulphate and *p*-toluenesulphonic acid showed a broad peak on the chromatogram.

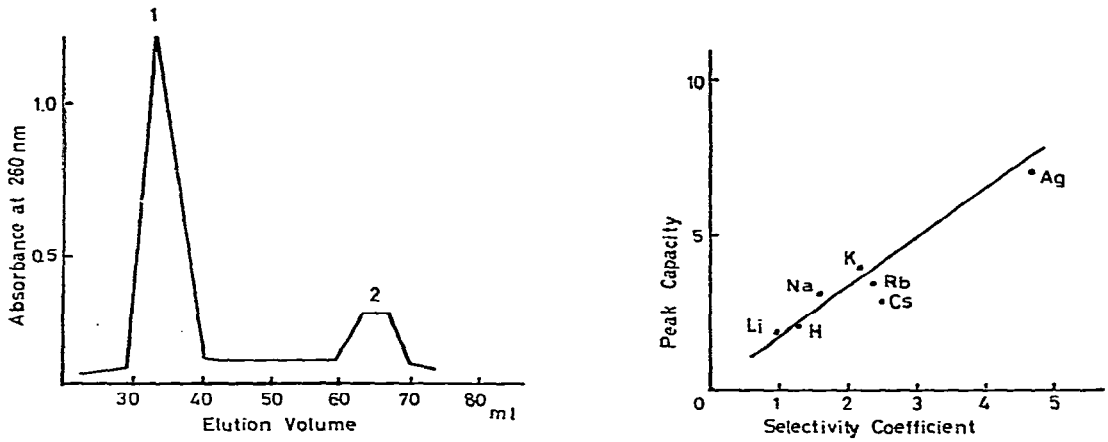


Fig. 3. Separation of *p*-toluenesulphonic acid lithium salt (1) and marinobufagin 3-sulphate potassium salt (2) on Sephadex LH-20. Solvent: methanol.

Fig. 4. Relationship between peak capacity of *p*-toluenesulphonic acid salt and selectivity coefficient of metal ion for the cation exchanger.

These results show that only univalent metal salts of an organic sulphate are distinctly separated by chromatography on Sephadex LH-20 with methanol. As illustrated in Fig. 4, there was a linear relationship between the peak capacity of *p*-toluenesulphonic acid salt and the selectivity coefficient of the metal ion for the cation exchanger[4], with the exception of the caesium salt. It is sufficiently substantiated that separation mechanisms other than molecular sieving, namely adsorption and ion exchange[5–7], are also operative in chromatography on Sephadex LH-20. The chromatographic behaviour of alkali metal ions that form ion pairs has previously been demonstrated[8]. This kind of ion-pair separation seems to be limited to alkali metals to methanol and ethanol–water mixtures.

Further studies on the chromatographic behaviour of biologically important steroid sulphates are being conducted in these laboratories.

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