PRELIMINARY CHEMICAL CHARACTERIZATION OF PHARMACOLOGICALLY ACTIVE COMPOUNDS OF AQUEOUS EXTRACTS OF SYNADENIUM GLAUCESCENS

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ABSTRACT: A test system based on the use of ion exchanges, gel filtration on Sephadex and extraction with organic solvents is described. This system provides information on the stability, molecular size, charge, and polarity of the pharmacologically active compounds in the aqueous plant extracts. Knowledge of these properties permitted the development of an isolation method for a glucose present in the aqueous extracts of the leaves of S.galucescens, responsible for the inhibition of the electrically induced contractions of a quinea pig ileum.

INTRODUCTION

Plant drugs have been used for centuries for the treatment of many diseases. Synadenium glaucescens which belongs to Eurphorbiaceae family, is a plant drug used in the preparation of various traditional medicines. No therapeutic use of literature. S. glaucescens has, however been reported as a poisonous plant of no therapeutic value (Verdcourt and Trump, 1969; Watt and Breyer-Brand Wijk, 1962).

In studying a plant used in traditional medicine, it is necessary to investigate aqueous extracts thoroughly because water is commonly used by medicine men as the extracting solvent. There are, however technical difficulties in the isolation of pharmacologically active compounds from aqueous extracts. The combination of appropriate chemical techniques out fractions with various pharmacological activities from aqueous extracts. The chemical procedures applied include test for stability, molecular size, basicity, acidity, polarity and acetone precipitation.

Testing stability of the aqueous extracts is very important. Aqueous solutions of the extract are heated alone and after addition of acid and alkali. The pharma-cological activities of these solutions are compared with those of a reference solution of the extract. Such comparison gives information about the stability of the active compounds.

In testing for molecular size, an aqueous solution of the extract, is passed through a column of sephadex G-25. The eluate is divided into two parts; one part coming out first and the other one later. The fractions collected contain the high molecular weight compounds while the ones appearing later contain the low molecular weight compounds. Polypeptides, polysaccarides
and big saponin molecules have been found to appear in the high molecular weight fractions.

Separation of compounds on their acidity or basicity is also an important chemical procedure. Aliquots of a solution of the extract are passed through a cation and anion exchanger (H+, OH-) and the pharmacological activity of the filtrate is tested. Loss of activity indicates absorption of active compounds on to the ion exchanger and thus implies basic or acidic properties of the active substances.

When testing for polarity, aliquots of an aqueous solution of the plant extract are extracted with the following solvents: n-butanol, ethylacetate, chloroform and diethylether. After removal of the organic solvent, the organic extract and the corresponding aqueous phase are tested for pharmacological activity.

Acetone precipitation is an important procedure. The aqueous solution of the extract is mixed with 10 volumes of acetone and the resulting precipitate collected by centrifugation. Following removal of the acetone from supernatant, both fractions are tested for pharmacological activity. If the activity is retained solely in the supernatant or in the precipitate, then this procedure would be useful as the first purification step in the isolation procedure.

RESULTS AND DISCUSSION

When all fractions have been tested for the appropriate pharmacological activity, enough information about the chemical properties of the active compounds is usually at hand to permit formulation of a suitable isolation procedure. Acetone precipitation is often a convenient isolation method if the pharmacological activity is completely retained either in the precipitate or in the supernatant. For charged compounds, ion exchanges are useful. The active material can be absorbed and all the impurities washed out of the column before elution of the desired compounds. The choice of the ion exchanger is dependent on the stability of the active compound. Acid or alkali labile substances should be adsorbed on to weak ion exchanger, permitting the use of milder conditions for elution.

For compounds of higher molecular weight, e.g sugar rich saponins or polypeptides, gel filtration is often useful. However, some brown coloured substances from plant extracts have a strong affinity for sephadex matrix. These compounds can be very difficult to wash out of the gel matrix. Gel filtration should therefore not be included in the first steps of the isolation procedure.

Employment of the described procedure, in combination with the guinea pig ileum has enabled us to devise isolation procedures of pharmacologically active compounds.

As an illustration of the use of the procedure, the identification of a glucoside in the leaves and stems of S. glaucescens, as a guinea–pig ileum contraction inhibitor is described. An aqueous extract of the stems and leaves was found to cause weak inhibition of electrically induced contractions of the guinea-pig ileum. The stability tests indicated that the active compound was stable to heat and treatment with acid or alkali. In the ion-exchange experiments the active fraction was found in the water eluate of the cation exchanger, indicating acidic or neutral properties. The activity was extracted into the aqueous phase when partitioned with organic solvents indicating a high degree of polarity. On acetone precipitation activity was retained in the precipitate.
These results indicated that the compound responsible for the inhibition activity on the guinea pig ileum was a stable, highly polar, low molecular weight, acidic compound stability. As all the activity was retained in the precipitate from acetone, this procedure was used as the first purification step. TLC experiments showed the compound to be a sugar derivative. This was supported by the test tube reaction involving phenol and sulphuric acid. Spectroscopic information obtained from 1HNMR and also detailed Cosy HNMR, plus 13 CNMR showed the compound to be a β-glucoside attached to a groups, methane, methylene and perhaps two more carbons in the structure. From a spectroscopic point of view the structure of the sugar and its attachment to the methane and methylene groups was confirmed. Studies are continuing to ascertain the position of the aglycone. Such studies involve methanolysis and other structural modifications.
PHARMACOLOGICAL ACTIVITY INVESTIGATED. INHIBITION OF ELECTRICALLY STIMULATED CONTRACTIONS OF THE GUINEA PIG ILEUM

Flow Chart 1: Fractionation of the pharmacologically active constituents of the aqueous extract of *S. glaucescens*
SELECTED FRACTIONATION OF THE PHARMACOLOGICALLY ACTIVE CONSTITUENTS OF AQUEOUS EXTRACT FROM *S. glaucescens*

Flow Chart 2. Selected fractionation of the pharmacologically active constituents of *sarsaparilla* extract from *S. glaucescens*. simple aglycone. The aglycone consists of two units.
Experimental

*S. glaucescens* stems and leaves were sent from the Traditional Medicines and Drugs Research Centre (KEMRI) to the Pharmacognosy laboratories, Uppsala, Sweden. The plant was identified at the Herbarium, National Museum, Kenya.

**Extraction**

1200 g of wet stems and leaves of *S. glaucescens* were cut using rotating knives in a blender. The material was extracted in 8 litres of water in the cold and stirred for 24 hours. The extract was filtered under vacuum and the filtrate evaporated in a cyclone distiller. The concentrated extract was then evaporated further on a rotary evaporator and lyophilized.

Mass of lyophilized extract was 41.884 g.

**Acetone Precipitation**

10 g of the water extract from *S. glaucescens* was precipitated in 1 litre of acetone. The supernatant and precipitate were collected. The precipitate was dried by evacuation and then lyophilized. Mass of lyophilized precipitate was 8.018 g.

**Ion Exchange Experiments**

160 ml of Dowex WX2 (H+, 50 – 100 mesh, Carl Roth, Karlsruhe F.R.G.) was stirred with 800 ml of 1N HCl for 30 minutes. The acid was filtered off and the ion exchanger washed with water until the eluate was colourless. The filtrate was collected, neutralized, concentrated *in vacuo* and lyophilized.

Mass of water eluate obtained from the cation exchanger was 5.007 g.

**n-butanol-water Partitioning**

5.007 g of the cation exchanged sample was dissolved in 300 ml of water and extracted with 1 litre of n-butanol (5 x 200 ml). The aqueous phase was concentrated *in vacuo* and lyophilized.

Amount of extract in aqueous phase was 4.757 g.

**Chromatography**

1. **Thin Layer Chromatography**

The aqueous phase obtained after partitioning with n-butanol was chromatographed on analytical precoated silica gel using the solvent system n-propanol: ethanol: acetic acid (10:50:20 (10%)). The spray reagent used was anisaldehyde-sulphuric acid. Compounds were separated that gave the following Rf values: blue spot (0.26), green spot (0.39), yellowish green spot (0.59), and another yellowish ‘green’ spot (0.70).

2. **Flash Chromatography**

The column was packed with silica gel (230 – 400 mesh ASTM) for column chromatography. 2.5 g of the sample from aqueous phase obtained after partitioning with n-butanol was introduced into the column and eluted with methanol: chloroform: acetic acid (85:5:10). 892.0 mg
of the ‘green’ compound with Rf 0.59 was obtained.

3. Chromatography on Sephadex LH20

100 mg of the ‘green’ compound was passed through sephadex LH20 which was pre-swollen overnight under water. The column was eluted using water and the eluate collected.

Mass of ‘green’ compound obtained was 56 mg.

Test for Sugar

Approximately 1 mg of the ‘green’ compound was dissolved in 1 ml of water in a boiling tube. 1 ml of phenol solution was added quickly followed by 5 ml of concentrated sulphuric acid and mixed. A red colour indicative of sugar was shown.

Isolated guinea pig ileum test (Rossel et al., 1977)

The terminal portion of the ileum was used after discarding the 5 cm nearest to the ileo-cacecal junction. The ileum was suspended in the Krebs solution (5 ml) bubbled with 95% O₂ and 5% CO₂ at a temperature of 36 – 37 degrees centigrade. Inhibitions were recorded by means of transducers (Grass FT03) and a Grass 79 polygraph equipped with pre-amplifier, main amplifier, oscillograph, and time and event marker. Histamine (200 ng | ml) was used for standardization.

Conclusion

The work described is in progress and more results will be presented in due course. Other components with pharmacological activity will be isolated. The techniques described above are useful when handling traditional medicines, nevertheless one has to exercise care not to misinterpret results at times. For example, when testing the molecular size the choice of a marker to dermacate low and high molecular weight compounds eluates has to be decided. It should also be noted that when dealing with ion exchangers one need compounds can be adsorbed onto the ion exchange matrix giving a false indication of the charge of the active substances.

Finally, it should be noted that the development of an isolation procedure for pharmacologically active compounds must be monitored by pharmacological tests. The observed activities must also be correlated with the yields of the active fractions, thus ensuring that no active compounds are lost or that their activity is not reduced as a result of unsuitable conditions in the isolation method. In this way the described approach for the isolation of the active compound has a big advantage since activity is monitored all through.

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