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REVERSE PHASE FLASH CHROMATOGRAPHY: A METHOD FOR THE RAPID PARTITIONING OF NATURAL PRODUCT EXTRACTS[1]

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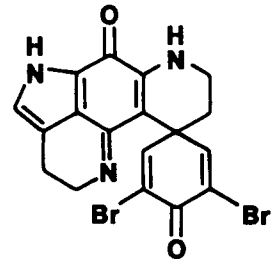
The separation of the complex matrix of compounds in any natural product extract presents a daunting problem to the natural product chemist. Although a variety of approaches are available to reduce the complexity of the problem, probably the most widely used method is that of liquid/liquid partitioning. A number of partitioning schemes are possible that rely on combinations of solvents for the separation of different classes of compounds. This technique can be used readily on quite large samples with no requirement for expensive equipment. Pettit *et al.* give a good example of a complex but effective liquid/liquid partitioning scheme developed over several years for one particular extract (1).

The discovery of potent antiviral and antitumor activities in certain New Zealand marine invertebrates has necessitated the partitioning of natural product extracts on a scale ranging from a few grams to more than a kilogram. Partitioning of these extracts by liquid/liquid extraction and the subsequent assaying of the resultant fractions for antiviral and/or antitumor activities indicated that an active component was often distributed over several fractions of quite different polarities. The liquid/liquid partitioning technique was clearly not sufficiently selective with these extracts.

As the compounds of interest in these marine invertebrate extracts tended to be polar, an alternative approach based on the use of reverse-phase chromatographic adsorbents was explored. The overall aims were to have a system that was fast, did not require expensive equipment, and yet would still be capable of partitioning the components of an extract with greater resolution than the usual liquid/liquid partitioning method. Shimizu has recently reviewed the problems presented by such extracts (2). He dismissed reverse-phase chromatography as an initial fractionation technique because of low sample capacity and the expense of commercial columns. However, the capacity of reverse-phase supports has been much underrated, and by the simple expedient of preparing reverse-phase supports (3) and using a low-pressure flash chromatography technique (4), both of these problems were overcome. The technique is illustrated by the isolation of the highly cytotoxic, water-soluble sponge pigment, discorhabdin C [**1**] (5).



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RESULTS AND DISCUSSION

The method that evolved is to coat the extract, containing compounds ranging from salts to hydrocarbons, on to a reverse-phase support. This can then be loaded, as either an aqueous slurry or a powder, onto a flash chromatography column that has been slurry-packed with the same support. Elution with H_2O, followed by a steep, stepped gradient

[1]Presented, in part, at the PAC CHEM 84 Congress, Honolulu, 16-21 December 1984.

through MeOH to CH_2Cl_2 generally gives very satisfactory partitioning of crude extracts. The recovery of material is usually very good.

In the example chosen, a strongly cytotoxic extract prepared from a *Latrunculia* species of sponge was partitioned. The results of the partitioning are shown in Table 1. Analysis of the fractions indicated that one compound, a red pigment, was present in all of the cytotoxic fractions. Medium-pressure reverse-phase liquid chromatography (rplc) of these combined fractions, followed by semi-preparative rplc, gave analytically pure discorhabdin C [**1**], the component responsible for the cytotoxicity of the crude extract. The structure of this novel compound was solved by a single-crystal X-ray diffraction study (5). Fraction 11 was largely sterols, of which the major component was cholesterol (^{13}C-nmr spectroscopy) while virtually all the salt was eluted in fraction 1.

When applied to other extracts from sponges, ascidians, and bryozoans, this method cleanly separated the components in the range from salts to sterols, lipids, and carotenoids, leaving them in a form ideally suited for further purification by semi-preparative rplc. As well as partitioning classes of compounds, this approach often resulted in the partial separation of components within given classes of compounds.

Using this method the sesterterpene tetronic acid variabilin was isolated from a number of sponges of the order Dictyoceratida (6); the highly oxygenated triterpene thyrsiferol and the various lauthisan eneynes from the red alga *Laurencia thyrsifera* (7,8); 1,3,7-trimethylguanine from the discorhabdins and sterols in New Zealand species of the sponge genus *Latrunculia* (9); oroidin from A-norsterols in a New Zealand species of the sponge genus *Axinella*.[2] Although we have only applied this technique to marine natural products, it is of wider general application.

The possible choice of solvents is wide, while the volumes used are minimal, thus lowering costs and enhancing ease of handling. The time taken for sample preparation and chromatography

TABLE 1. Reverse-Phase Column Fractionation of Sponge Extract

Fraction No.	Eluent	Volume (ml)	Mass (mg)	Activity[a]
1	H_2O	100	6746	−
2	H_2O	100	72	+/−
3	H_2O-MeOH (3:1)	100	45	−
4	H_2O-MeOH (3:2)	100	318	+/−
5	H_2O-MeOH (2:3)	100	191	++
6	H_2O-MeOH (1:4)	100	83	+
7	H_2O-MeOH (1:9)	100	63	++
8	MeOH	100	108	++
9	MeOH-CH_2Cl_2 (9:1)	100	90	+
10	MeOH-CH_2Cl_2 (3:1)	100	98	−
11	MeOH-CH_2Cl_2 (1:1)	100	515	−
12	CH_2Cl_2	200	604	−
13	MeOH+H_2O	400	154	−

[a]Cytotoxicity to BSC cells in 16-mm wells with a loading of 100 ng/6-mm disk.
++: 2-4 mm zone.
+: 1-2 mm zone.
+/−: minor effects located under disk.

[2]Unpublished results, J.W. Blunt and M.H.G. Munro.

is typically about 3 h. The equipment used is inexpensive, and the reverse-phase support may be re-cycled many times, thus offsetting the high initial cost of commercial support. A cheaper alternative, which we have used, is to prepare appropriate reverse-phase packing materials from chromatographic grade silica (3). Finally, this is a high capacity system capable of handling up to about 20 g of crude extract per 100 g of support.

EXPERIMENTAL

MATERIAL.—Reverse-phase material was prepared by coating Si gel (Woelm, 32-63 microns) with *n*-octadecyltrichlorosilane by the method of Evans *et al.* (3).

METHOD.—The MeOH/toluene extract (9.7 g) of a sponge of the genus *Latrunculia*, family Latrunculiidae (voucher no. T430-8) was dissolved/suspended in CH_2Cl_2/MeOH/H_2O (250 ml) and added to the reverse-phase material (5 g). Concentration under reduced pressure gave an aqueous slurry (50 ml) that was added to the top of a column (25 mm i.d.) which had been slurry packed with the same material (50 g) in MeOH and then equilibrated with H_2O. Details of eluents and fraction volumes are given in Table 1. Each fraction was assayed for antiviral/cytotoxic activity (10), but using a BSC cell line rather than CV-1 cells and examining each well microscopically for cytopathic and cytotoxic effects.

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