Table I—Average (±*SD*) Hardness of Tablets in Kilopounds at Various Temperatures (±1°)

Product	−25°	0°	24°	50°
A[a]	9.7 ± 1.3[b]	8.2 ± 1.2	9.0 ± 0.9	7.1 ± 0.9
B[c]	6.7 ± 1.1	6.3 ± 1.1	6.0 ± 1.2	5.0 ± 1.4
C[d]	7.5 ± 0.8	6.5 ± 0.6	6.6 ± 0.6	4.5 ± 0.4
D[e]	7.1 ± 2.4	6.5 ± 2.2	6.7 ± 1.7	5.8 ± 1.3
E[f]	7.4 ± 0.7	6.6 ± 0.8	7.0 ± 1.0	6.0 ± 1.0
F[g]	7.2 ± 0.8	8.6 ± 0.8	9.0 ± 0.8	6.2 ± 0.5
G[h]	15.3 ± 2.2	13.6 ± 2.2	11.2 ± 1.1	5.0 ± 0.6

[a] Belladenal, Sandoz, lot 241 A 8473 (July 1984). [b] Average of 10 measurements. [c] Furadantin (50 mg), Norwich-Eaton, lot 02276 (August 1984). [d] Hydergine, Sandoz, lot 764 Y 6460 (July 1981). [e] Methotrexate, Lederle, lot 513-418 (May 1982). [f] P-A-C Compound, Upjohn, lot 670GK (March 1982). [g] PBZ Lontabs (50 mg), lot 11172 (September 1981). [h] Tedral SA, Warner-Chilcott, lot 2210V075A (July 1980).

Table II—Average (±*SD*) Hardness of Tablets in Kilograms[a] at Various Temperatures (±1°)

Product	−25°	0°	24°	50°
H[b]	19.9 ± 0.4[c]	19.7 ± 0.3	19.4 ± 0.6	20.0 ± 0.2
I[d]	19.8 ± 0.4	19.3 ± 1.5	17.9 ± 1.9	20.2 ± 0.3
J[e]	19.3 ± 0.4	19.9 ± 0.2	18.5 ± 0.6	19.6 ± 0.6

[a] Pfizer hardness tester. [b] Gris-PEG, Dorsey, lot L77207 (February 1981). [c] Average of 10 measurements. [d] Tenuate Dospan, Merrell, lot 155BB (January 1984). [e] Tral Gradumet, Abbott, lot 53988AF26 (April 1980).

exist to measure or express the hardness of compressed tablets, hardness is a universally used manufacturing specification for inprocess control and batch evaluation. The embrittlement of compressed tablets at temperatures (−25–50°) likely to be encountered in handling, shipment, and storage is of interest.

EXPERIMENTAL

Ten commercial products were arbitrarily selected without regard to the medicinal compound. The tablets were placed in separate amber glass vials at ambient temperature and humidity. Tablets were stored in a freezer at −25° for 20 hr, in an oven at 50° for 20 hr, or in an ice bath at 0° for 8 hr. Tablets were removed from the constant-temperature chambers and immediately measured by means of a motor-driven hardness tester[1], which applied force at a constant rate. A manual hardness tester[2] was used only when the hardness exceeded the scale on the motor-driven tester. For each product, 10 tablets were measured.

RESULTS AND DISCUSSION

The mean hardness and standard deviation of seven commercial tablets at −25, 0, 24, and 50° are shown in Table I. The mean hardness and standard deviation as determined by the manual hardness tester permits comparison of hardness at various temperatures (Table II).

Inspection of Table I suggests a slight decrease in hardness as the temperature was increased (~−0.02 kilopound/degree) for Products A–E. The sustained-release tablets (F and G) were softest at the highest temperature. Wax-like ingredients (carnauba wax and stearyl alcohol), which impart a sustained-release pattern, probably soften and become more plastic as the temperature rises from room temperature to 50°; this effect decreased the hardness by 50% for Tablet G. Since the exact formulations are unknown, speculation on the form of the curves serves no useful purpose. However, the results confirm that for nonconventional (sustained-release) tablets, one would not necessarily anticipate a constant value of the thermal hardness coefficient as was demonstrated for the conventional tablets.

In the temperature range studied, Products H–J had a thermal hardness coefficient of zero (Table II). In these products, the medicinal compound is held within a matrix. For example, in Product J, the hexocyclium methylsulfate exists within the channels and pores of a water-insoluble matrix of methyl acrylate and methyl methacrylate, which maintains a constant hardness at the usual temperatures of handling and storage.

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[1] Schleuniger model 2E/205, Vector Corp., Marion, IA 52302.
[2] Pfizer Co., New York, NY 10017.

Dry Column Chromatographic Procedure for Rapid Concentration of Biological Activity in Natural Products Fractionation

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Abstract □ A dry column chromatographic procedure is described. It allows for the rapid concentration of biologically active materials in natural products fractionation. The potential value of the technique is described, utilizing as an example the separation of an anticancer active fraction obtained from *Euphorbia cyparissias*.

Keyphrases □ Column chromatography, dry—rapid concentration of biological activity, natural products fractionation □ *Euphorbia cyparissias*—separation of anticancer active fraction, dry column chromatography □ Separation—biological activity in natural products, dry column chromatography

One of the largest and most successful plant screening programs in recent years has been coordinated by the U.S. National Cancer Institute to discover new naturally occurring tumor inhibitors (1–3). As part of this effort, this laboratory established an extensive program to screen the higher plants of southeastern Michigan for anticancer activity and to isolate active plant constituents. As with all work of this type, the successful completion of fractionation studies depends largely on the rapid concentration of active materials into simplified fractions so that maximal effort can be directed toward purification of active plant components. To facilitate this process, a method was sought that would rapidly indicate which plant constituents were responsible for activity observed with crude plant extracts.

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Table I—Separation Results and Biological Activity of Fractions Obtained from Dry Column Chromatography of *E. cyparissias* Chloroform Fraction

Fraction	Band Position[a], cm	Weight[b], mg	Elution Solvent[c]	PS Activity, best % T/C (Dosage, mg/kg)	KB Activity, ED_{50}, μg/ml
Ethanol extract	—	—	—	147 (12.5)	30
Chloroform fraction	—	—	—	141 (4)	7.5
A	Eluted	43	Benzene–hexane (1:1)	105 (1)	70
B	0–8	40	Benzene–hexane (3:1)	109 (2)	70
C	8–12	27	Benzene	108 (4)	2.2
D	12–24	54	Benzene–chloroform (1:1)	100 (2)	23
E	24–28	20	Benzene–chloroform (15:85)	123 (0.5)	17
F	28–40	48	Chloroform	115 (2)	29
G	40–44	60	Chloroform	115 (2)	26
H	44–56	27	Chloroform–methanol (98:2)	135 (1)	28
I	56–60	189	Chloroform–methanol (9:1)	119 (4)	70

[a] Position of band from bottom of a 60-cm column. [b] Amount recovered from a 525-mg sample. [c] Solvent required to obtain similar materials *via* gradient-elution column chromatography on a separate 9-g sample.

Various methods have been described for the fractionation of anticancer active plant extracts (3, 4). Unfortunately, conventional techniques may require considerable time and effort before work can be focused on active fractions. One method that has not been utilized extensively in natural products fractionation is dry column chromatography, although procedures have been available (5–8). Dry column chromatography usually is employed to scale-up preparative TLC procedures in the final purification of materials. Because dry column chromatographic procedures can be performed in one afternoon, the method is ideal for initial fractionation. Complex mixtures may be separated into 10 or more fractions whose bioassay may provide a clue to the material responsible for the observed anticancer activity. Larger scale procedures then may be utilized to prepare sufficient quantities of the active fraction for purification and structural analysis.

The described dry column procedure has been successfully employed in the preliminary analysis of several plants including *Euphorbia cyparissias* L. (Euphorbiaceae), which is presented here as an example[1].

EXPERIMENTAL

Materials and General Procedures—TLC was performed on precoated silica gel plates[2], developed in chloroform–ether (95:5) and visualized by spraying with 3% ceric sulfate in 3 *N* H_2SO_4, followed by heating at 110° for 10 min. Dry column chromatography utilized silica gel[3] packed into 4-cm (flat) nylon tubing[4]. Silica gel activity on the Brockmann scale (9) was analyzed using *p*-dimethylaminoazobenzene[5] according to established procedures (6). Column chromatography was performed on silica gel 60[6] (70–230 mesh). Plant material[7] was collected from selected areas of southeastern lower Michigan, air dried, and milled to a fine powder.

Extraction and Preliminary Fractionation—Dried and milled *E. cyparissias* (all plant parts), 520 g, was macerated in ethanol (95%) at room temperature for 24 hr. Following filtration of the plant material and concentration of the filtrate *in vacuo*, the ethanolic extract (97.4 g) was partitioned between equal volumes of chloroform and water. Separation of the organic layer and concentration *in vacuo* yielded 34.3 g of a dark-green tar (chloroform fraction).

Dry Column Chromatography—An 80-cm length of nylon tubing was sealed at one end by folding the tubing several times and stapling. Holes were punched above the seal, allowing for drainage and escape of air during column packing, and a small pad of glass wool was inserted above the holes. Silica gel (175 g) was added in six or seven portions, and the column was compacted by sharply tapping or vibrating the sides while firmly grasping the column just above the adsorbent layer. Following addition of the final portion of adsorbent and compacting as before, the top of the column was uniformly compressed by inserting an inverted stopper (of the same approximate diameter as the column) attached to a glass rod and exerting pressure on the top of the column.

The sample to be chromatographed, 525 mg of the chloroform fraction, was dissolved in chloroform and added to 2 g of diatomaceous earth[8]. The resulting mixture, evaporated to dryness *in vacuo*, was ground to a powder in a mortar and added to the top of the column. Following compression of the sample layer from the top, the column was completed by the addition of a 2–3-cm layer of sand. The prepared column, measuring ~2.5 × 60 cm, was supported by inserting a piece of open glass tubing into the open end and clamping the glass and nylon tubing together. Two additional clamps were added further down the column for support.

Column development was effected by adding the developing solvent (chloroform–ether, 95:5) under a constant liquid head of 3–5 cm. Separation of pigmented bands was observed within the first 10–15 cm, with further separation achieved as development continued. Additional solvent was added until yellow pigmented materials, moving with the solvent front, eluted from the column. The total development time was ~45 min, requiring 450 ml of solvent.

Immediately upon completion of column development, the column was laid on its side and sliced into 15 4-cm bands. Following extraction of adsorbent fractions with ether–methanol combinations, filtration, and concentration of the filtrates *in vacuo*, the extracted materials were compared by TLC and similar fractions were combined. Separation results and data from subsequent biological assay[9] are summarized in Table I.

Gradient Elution Column Chromatography—A 9-g sample of the chloroform fraction was chromatographed on 450 g of silica gel, eluting with combinations of hexane, benzene, chloroform, and methanol. A total of 165 fractions (125 ml each) was collected and combined on the basis of TLC behavior. Solvents required to elute materials similar to dry column chromatographic fractions are indicated in Table I.

RESULTS

As indicated in Table I, an ethanolic extract of *E. cyparissias* possessed activity in the P-388 (PS) lymphocytic leukemia *in vivo* assay in mice (an active fraction possesses a percent T/C value of ≥130). Following solvent partitioning between chloroform and water, the activity was shown to reside in the chloroform fraction, which also demonstrated activity in the 9KB carcinoma of the nasopharynx *in vitro* assay (activity is indicated by ED_{50} values of ≤20 μg/ml for crude fractions and of ≤4 μg/ml for purified materials). TLC analysis of the active chloroform fraction indicated

[1] Complete details concerning *E. cyparissias* fractionation and structural work on isolated materials will be published.
[2] Analtech.
[3] Woelm Pharma, Eschwege, West Germany.
[4] Universal Scientific.
[5] Aldrich Chemical Co.
[6] E. Merck, Darmstadt, West Germany.
[7] The plant material was identified as *Euphorbia cyparissias* L. (Euphorbiaceae) by Dr. C. M. Rogers, Department of Biology, Wayne State University. A voucher specimen (No. 16218) is preserved in the Wayne State University Department of Biology Herbarium.
[8] Celite, Fisher Scientific Co.
[9] Extracts and fractions were tested under the auspices of the Developmental Therapeutics Program, National Cancer Institute (10).

it to be a complex mixture, containing a minimum of 20 components. No information was available to indicate which component was responsible for the observed activity.

The results of dry column separation of the chloroform fraction indicated the presence of at least two active materials (Table I), a higher R_f fraction (Fraction C) possessing *in vitro* KB activity and a more polar fraction (Fraction H) possessing PS activity. While certain additional fractions possessed marginal KB activity, subsequent fractionation efforts were directed at the two most active materials. With biological data available from dry column chromatographic analysis, larger scale gradient-elution column chromatographic procedures could be aimed specifically at the resupply of the active materials.

DISCUSSION

The complex nature of the biologically active *E. cyparissias* chloroform fraction presented a formidable challenge to the dry column chromatographic technique. Although all components of this mixture were not separated completely (some fractions contain more than one component), useful separation was achieved and, on the basis of dry column chromatographic results, subsequent efforts could be directed toward a limited group of materials possessing bioactivity. The value of the dry column technique in the preliminary analysis of a complex active fraction thus was demonstrated. In examples not presented here, pure materials could be isolated and their activity determined directly from the dry column procedure.

In certain investigations involving the fractionation of biologically active plant or fermentation extracts, a previously isolated active material may be presumed to be responsible for the observed activity based on phytochemical or chemotaxonomic information. Much effort may be saved in these instances by employing the dry column chromatographic procedure as the initial fractionation step. If the R_f of the material is known, the corresponding dry column chromatographic band can be obtained and analyzed for activity as well as for the presence of the known compound.

As indicated in Table I, a developing solvent for dry column chromatographic analysis can be utilized that will produce results roughly similar to those obtained from extensive column chromatographic procedures. By employing dry column procedures first, however, careful liquid chromatographic techniques need only be employed to separate components of active fractions, allowing inactive materials to be collected together. Thus, some time, effort, and expense of conventional techniques may be saved.

The described dry column technique represents only minor modification of previously described dry column chromatographic techniques. Although such methods have not been heavily utilized by the natural product chemist, they should become increasingly important in improving the efficiency and speed of fractionation efforts.

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Absorption of Orally Administered Sodium Sulfate in Humans

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Abstract □ Sodium sulfate can be used to enhance the conjugation of phenolic drugs with sulfate and to treat hypercalcemia. It is thought that sulfate ion is absorbed slowly and incompletely from the digestive tract. The purposes of this investigation were to determine the absorption of a large amount of sodium sulfate (18.1 g as the decahydrate, equivalent to 8.0 g of the anhydrous salt) and to compare the bioavailability when this amount is administered orally to normal subjects as a single dose and as four equally divided hourly doses. The 72-hr urinary recovery of free sulfate following single and divided doses was 53.4 ± 15.8 and 61.8 ± 7.8%, respectively (mean ± *SD*, $n=5$, $p > 0.2$). The single dose produced severe diarrhea while the divided doses caused only mild or no diarrhea. Thus, a large amount of sodium sulfate, when administered orally in divided doses over 3 hr, is well tolerated and is absorbed to a significant extent. Orally administered sodium sulfate may be useful for the early treatment of acetaminophen overdose.

Keyphrases □ Sodium sulfate—absorption after oral administration, single and divided doses, bioavailability, humans □ Acetaminophen—use of sodium sulfate for early treatment of toxicity, absorption of orally administered sodium sulfate, single and divided doses □ Bioavailability—sodium sulfate, comparison of single and divided doses, role in treatment of acetaminophen toxicity

Humans and animals have a limited capacity to conjugate phenolic drugs with sulfate (1–5). The limiting factor is the availability of sulfate ion rather than its activation or the transfer of activated sulfate to the acceptor molecule (3, 5). Sulfate ion is acquired by the body partly as such from dietary sources and partly by oxidation of cysteine and methionine (6). The possibility of enhancing the formation of phenolic sulfates by direct administration of inorganic sulfate was first proposed in 1876 and has been demonstrated by several investigators (6, 7). Typically,

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