**Table III—*In Vitro* Effects of Imide Analogs on Enzymes of Cholesterol and Triglyceride Synthetic Pathways in Mice ($n$ = 6)**

| Compound | Percent of Control, $\bar{x} \pm SD$ | | | |
|---|---|---|---|---|
| | Acetyl-CoA Synthetase | Citrate-lyase | Acetyl-CoA Carboxy-lase | Fatty Acid Synthetase |
| I | 70 ± 8[a] | 42 ± 6[a] | 8 ± 4[a] | 105 ± 8 |
| II | 53 ± 12[a] | 34 ± 4[a] | 17 ± 3[a] | 109 ± 7 |
| III | 74 ± 6[a] | 38 ± 3[a] | 87 ± 7 | 98 ± 9 |
| IV | 58 ± 6[a] | 38 ± 7[a] | 100 ± 5 | 81 ± 7[b] |
| V | 63 ± 9[a] | 66 ± 6[a] | 106 ± 6 | 86 ± 8 |
| VI | 88 ± 5[b] | 60 ± 5[a] | 59 ± 8[a] | 103 ± 4 |
| VII | 61 ± 7[a] | 65 ± 6[a] | 9 ± 2[a] | 93 ± 5 |
| VIII | 74 ± 9[a] | 47 ± 8[a] | 12 ± 3[a] | 104 ± 7 |
| IX | 57 ± 10[a] | 87 ± 6[b] | 18 ± 4[a] | 107 ± 7 |
| X | 87 ± 8[b] | 82 ± 7[a] | 54 ± 5[a] | 110 ± 8 |
| XI | 58 ± 7[a] | 76 ± 4[a] | 24 ± 4[a] | 91 ± 9 |
| XII | 44 ± 11[a] | 72 ± 9[a] | 76 ± 3[a] | 75 ± 10[b] |
| Acetazolamide | 66 ± 4[a] | 66 ± 7[a] | 13 ± 2[a] | 82 ± 6[b] |
| Carboxymethyl-cellulose, 1% | 100 ± 5[c] | 100 ± 4[d] | 100 ± 7[e] | 100 ± 7[f] |

[a] $p \leq 0.001$. [b] $p \leq 0.005$, Student $t$ test. [c] 28.5 mg of acetyl-CoA formed/g of wet tissue/30 min. [d] 30.5 mg of citrate hydrolyzed/g of wet tissue/30 min. [e] 32,010 dpm/g of wet tissue/30 min. [f] 37,656 dpm/g of wet tissue/30 min.

0.001). These agents were not toxic at the doses employed, and no side effects were noted.

An attempt was made to correlate the antihyperlipidemic activity with the ability to inhibit enzymatic activities at key biochemical sites early in the synthesis of cholesterol and fatty acids (Table III). The ability to lower serum cholesterol levels correlated positively with the ability to suppress liver acetyl-CoA synthetase activity ($r = 0.86$, $p = 0.001$, using the means of each test group[7]). The ability to suppress acetyl-CoA carboxylase activity correlated positively with the lowering of serum triglycerides ($r = 0.84$, $p = 0.001$). The ability to inhibit citrate-lyase activity, although inhibited by I–IV and VIII which caused >50% inhibition, did not correlate with the ability to lower the serum cholesterol or triglycerides levels. The imide derivatives had no effect on fatty acid synthetase activities except in isolated cases such as IV, V, and XII. Both acetyl-CoA synthetase and citrate-lyase regulate the availability of acetyl-CoA in the cytoplasm for the synthesis of both cholesterol and fatty acids for the synthesis of triglycerides. Acetyl-CoA carboxylase is the regulatory enzyme in the synthesis of fatty acids, which subsequently are required in triglyceride synthesis. In this assay, the carboxylase enzyme must be polymerized for optimum activity (10). Highest inhibition by the imide compounds was observed when test compounds were added to the incubation medium prior to polymerization. However, significant inhibition was observed even if the compounds were added after polymerization, *e.g.*, I, II, VIII, and IX caused 76, 62, 42, and 70% inhibition of the carboxylase activity, respectively.

These studies demonstrate pellucidly the antihyperlipidemic effects of imides. Compared to available standard pharmacological agents, these agents are potent in their ability to lower serum lipids at a relatively low dose with no observable deleterious side effects. These compounds offer unique ways to regulate lipid synthesis which have not been reported previously.

[7] Pearson-product-moment coefficient of correlation ($r$); probability determined by the Student $t$ test (12).

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# Thermal Hardness Coefficient of Tablets


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Received May 7, 1980, from the *Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication September 2, 1980.



**Abstract** □ The hardness of 10 commercial compressed tablets was measured at −25, 0, 24, and 50°. The hardness is relatively insensitive to temperature change within normal storage and handling temperatures. Consequently, no temperature control is needed in measuring tablet hardness. Nonconventional (sustained-release) tablets behave differently.



**Keyphrases** □ Tablets—determination of hardness at various temperatures □ Hardness—tablets, effects of temperature □ Temperature—effect on hardness coefficient of tablets


Carbon steel and martensitic steels have low mechanical strength at low temperatures. With the exception of tetrafluoroethylene resin, plastics are embrittled at low temperatures (1). In the cryopulverizing process, the material to be comminuted has its temperature lowered so that it changes from a ductile to a brittle solid (2, 3). All materials are not embrittled by chilling; copper, aluminum, nickel, and most solid–solution alloys of these metals are strong at low temperatures.

In pharmaceutics, the brittleness or resistance to crushing is known as hardness, which is defined as the compression force that, when applied diametrically, just causes the tablet to fracture. Although no official standards




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

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Received June 13, 1980, from the *College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI 48202.* Accepted for publication August 28, 1980.

**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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