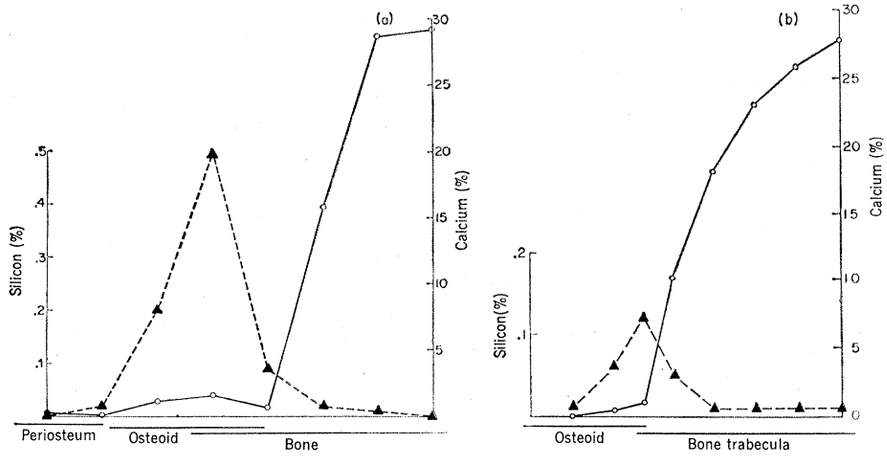

Fig. 1. Spatial relation between silicon (▲) and calcium (○) composition (percent, by weight) in typical traverses across young tibia in (a) a periosteal region (cross section) and in (b) a metaphyseal trabecula (longitudinal section) as obtained by electron-microprobe techniques.

were performed by moving the specimen in 3- to 4-μm steps under a stationary electron beam focused to a diameter less than 2 μm.

The spatial distribution of the changing calcium and silicon compositions in young bone is shown in Fig. 1a, representing one of a great many traverses through sites rich in silicon in the periosteal regions. In the periosteum both the calcium and silicon values are invariably low, whereas in the adjacent osteoid layer of this sample (Fig. 1a), there are silicon-rich sites containing up to 25 times as much silicon and nine times as much calcium as in the periosteum. The silicon content falls again to the original extremely low value as the calcium concentration rises to 15 percent and beyond. Several other traverses across periosteal regions of this sample yield similar results.

A similar relation exists between calcium and silicon in the metaphysis of young bone, the presence of silicon corresponding with the margins of trabeculae and bony spicules in the course of formation. Part of a typical traverse across the metaphyseal region of a longitudinal section of a young tibia (Fig. 1b) shows silicon content rising from 0.01 to 0.06 percent in the preosseous border and to 0.12 percent on the edge of a trabecula but then declining to and remaining at 0.01 percent while calcium content rises progressively from 0.06 to 27.8 percent. This same relation was encountered eleven more times in the continuation of the traverse, not shown in the figure. Calcium contents in the silicon-rich sites, which are abundant in this part of bone, range from 0.15 to 0.70 percent and molar ratios of calcium to silicon range from 1/3 to 3. By comparison, on the edge of the trabeculae where the calcium content is about 1 percent, the molar ratios of calcium to silicon are typically about 5. The decline in silicon, as the traverse progresses into the trabecula, occurs usually at a calcium concentration above 1 percent. Silicon continues to fall and remains at a minimum as the calcium concentration progressively increases with distance from the edge of the trabecula.

The overall relation between silicon and calcium contents, in regions where silicon is found, in both periosteal and endochondral bone can be summarized as follows. At extremely low concentrations of tissue calcium (less than 0.1 percent) little or no measurable silicon is present. Silicon appears where a low to moderate and varying calcium content suggests active calcification. The silicon is not uniformly distributed in these areas but is concentrated in sites containing from 0.08 to 1.0 percent or more silicon and from 0.1 to 2.0 percent calcium on the edge of trabeculae or from 0.5 to about 15 percent calcium in the periosteal areas. Such sites are common. Generally a marked decline in silicon-rich sites occurs at calcium concentrations of 10 to 20 percent, and little or no measurable silicon is present at calcium concentrations above 20 percent, whether in areas of active calcification or not.

To help eliminate the possibility that the silicon increment could be a residual effect as tissue fluid space is replaced by apatite, simultaneous analyses were made in specific areas for silicon and for chloride, an anion not believed to participate in the calcification process (*1*). No correlation with the silicon effect was observed. However, a positive correlation has been established with magnesium, which has been suggested as a factor regulating mineralization in vivo (*3*).

These observations, relating to active bone development, imply that silicon may be allied to the initiation of mineralization of preosseous tissue, whether in periosteal or in endochondral ossification. Further evidence that silicon is involved in calcification at an early stage is provided by molar ratios of calcium to phosphorus. In hydroxyapatite, and in mature bone generally, this ratio is approximately 1.67. In the silicon-rich sites, even though calcium contents range appreciably, these molar ratios are typically in the range 0.6 to 0.8. Silicon-rich sites have yet to be found in bone approaching the composition of hydroxyapatite. Additional information from studies in vivo support the finding that silicon is associated with calcium in the mineralization process (*4*).

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5. This research was made possible through use of the electron microprobe facilities at the Department of Geology, UCLA, supported in part by NSF grant GA-1503 and in part by the General Research Support grant (FR 05442) of the School of Public Health, UCLA.

30 October 1969

Dimethylpropynylbenzamides: A New Group of Herbicides

Abstract. N-*(1,1-Dimethylpropynyl)-3,5-dichlorobenzamide is representative of a group of benzamides that are herbicidally active on annual and perennial grasses with potential agricultural utility in forage legumes, certain turf grasses, and cultivated crops.*

Several dimethylpropynylbenzamides have shown outstanding activity as selective herbicides. Preeminent among these is *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (Fig. 1), extensively field tested under the code designation RH-315.

This benzamide was prepared by

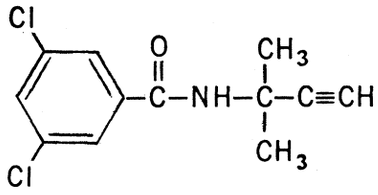

Fig. 1. *N*-1,1-(Dimethylpropynyl)-3,5-dichlorobenzamide.

adding one equivalent of 3,5-dichlorobenzoyl chloride dropwise, with stirring at 20°C, to an equimolar mixture of 3-amino-3-methylbutyne and 25 percent aqueous sodium hydroxide in Esso octane (boiling point 102° to 113°C). After 3 hours the white slurry was filtered, and the solid was washed with water and dried in a vacuum. The yield was 97 percent of a material that was 95 percent pure, as determined by gas-liquid partition chromatography. Two recrystallizations from aqueous methanol produced white needles with a melting point of 155° to 157°C. The spectral characteristics are: infrared (mull) 1650 cm^{-1} (C=O) and 3310 cm^{-1} (N–H and CH); nuclear magnetic resonance (in ppm) ($CDCl_3$) 1.75 (*s*, 6, C–C*H*$_3$), 2.40 (*s*, 1, C–*H*), 6.49 (*s*, 1, N–*H*), 7.46 (*t*, 1; J = 2 hz, C(4)-aromatic *H*), and 7.62 (*d*, 2; J = 2 hz, C(2,6)-aromatic *H*). The elemental analysis showed (percent) C, 56.42; H, 4.39; N, 5.46. Calculated values for $C_{12}H_{11}Cl_2NO$ are: C, 56.27; H, 4.33; N, 5.47.

Other herbicidally active dimethylpropynylbenzamides include the 3,5-dimethyl-, 3.5-dibromo-, 3.5-difluoro-, and 3-methyl-5-chloro- analogs. These compounds are toxic to seedlings and established plants of many annual and perennial species of the grass family, including quackgrass (*Agropyron repens*), and to many annual species in other families. However, it causes little or no injury to most legumes, including alfalfa (*Medicago sativa*), clovers (*Trifolium* sp.), trefoil (*Lotus* sp.), soybeans (*Glycine max*), beans (*Phaseolus* sp.), and peas (*Pisum* sp.); to cotton (*Gossypium* sp.); and to composites, including lettuce (*Lactuca sativa*), sunflower (*Helianthus annuus*), and safflower (*Carthamus tinctorius*).

Susceptible perennial grasses are killed by application of granules or sprays when adequate moisture is available to move the compound into the root zone. Little or no toxicity occurs from absorption by foilage. Examples of potential utility for these compounds include weed control in established forage legumes such as alfalfa, clover, and trefoil; weed control in lettuce; and the selective preemergence or postemergence control of annual bluegrass (*Poa annua*) and ryegrass (*Lolium perenne*) in turfs consisting of bermudagrass (*Cynodon dactylon*), St. Augustinegrass (*Stenotaphrum secundatum*), or zoysiagrass (*Zoysia* sp.). Available evidence indicates that use of *N*-(1,1-dimethylpropynyl) - 3,5 - dichlorobenzamide will not result in a toxic residue to rotation crops.

The acute oral LD_{50} of *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide is 8350 mg/kg for male albino rats, 5620 mg/kg for female albino rats, and > 10,000 mg/kg for mongrel dogs. The acute dermal LD_{50} for albino rabbits is > 3160 mg/kg.

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Countercurrent Chromatography: Liquid-Liquid Partition Chromatography without Solid Support

Abstract. *The liquid-liquid partition chromatographic system reported here involves a long helix of narrow-bore tubing. When the coiled tube is filled with one phase of a two-phase system and fed with the other phase, phase-interchange takes place in each turn of the coil, leaving a segment of the former phase as the stationary phase. Consequently, solutes present in either phase are subjected to a multistep partition process. The column efficiency, estimated on a separation of dinitrophenyl amino acids, is comparable to that of gas chromatography.*

Interaction of the solid support with the solute in conventional liquid-liquid partition chromatographic separation methods frequently causes "tailing" or denaturation of the solute. To overcome these problems, Ito *et al.* (*1*) have used the planetary motion of a helical tube in a centrifugal field to provide a two-phase liquid partition system that did not require a solid support. In the system reported here we use flow through a helical tube, rather than planetary motion, to move one phase with respect to the other. The principle of the technique is illustrated in Fig. 1. A horizontal helical tube is filled with the heavier phase of a two-phase system (Fig. 1a). The light (moving) phase introduced from one end pushes the heavy (stationary) phase until the light phase can rise over the heavy phase at the bottom of the coil (Fig. 1b). The process is repeated at each turn until the entire column is made up of half-turn segments of each phase (Fig. 1c). Continued introduction of the light phase displaces only the light phase so that solute introduced with either phase will be percolated through successive segments of the heavy phase and will be separated according to their relative partition coefficients, in a manner analogous to that of conventional liquid-liquid chromatography but in the absence of a solid support. Either the light or heavy phase may be the moving phase.

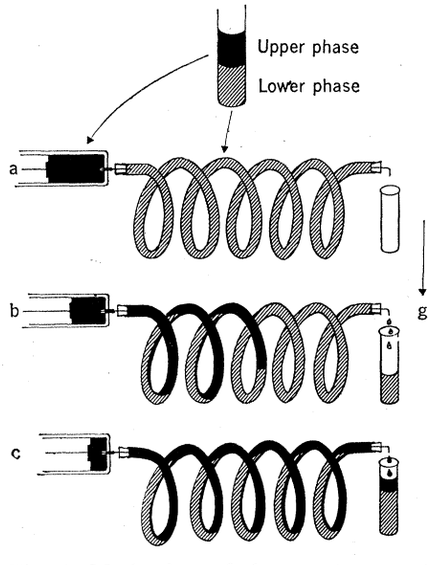

Fig. 1. Mechanism of the countercurrent process.

Columns of several thousand turns are easily made if the helix is of the order of 1 to 5 mm with tubing measuring less than 0.5 mm in inner diameter. The light phase fails to rise through the heavy phase in small capillaries unless phase separation is strengthened by centrifugal force. Coiling the helix support inside a Helixtractor head (International Equipment Company) and spinning the system at 100*g* pro-