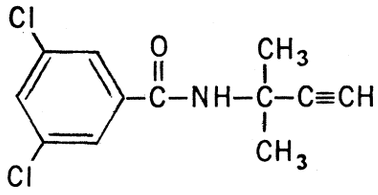

Fig. 1. *N*-1,1-(Dimethylpropynyl)-3,5-dichlorobenzamide.

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.

K. L. VISTE
Research Laboratories,
Rohm and Haas Company,
Spring House, Pennsylvania 19477
A. J. CROVETTI
B. W. HORROM
Scientific Divisions,
Abbott Laboratories,
North Chicago, Illinois 60064

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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.

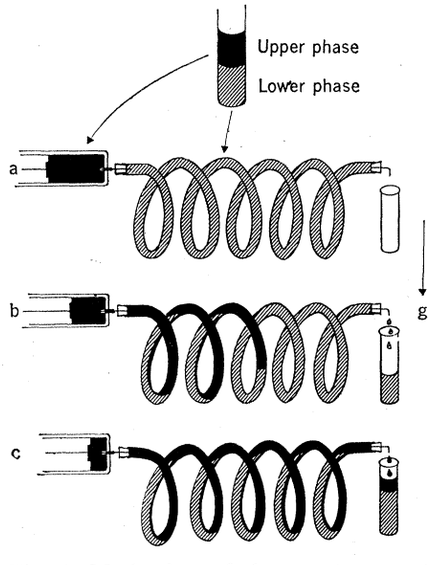

Fig. 1. Mechanism of the countercurrent process.

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-

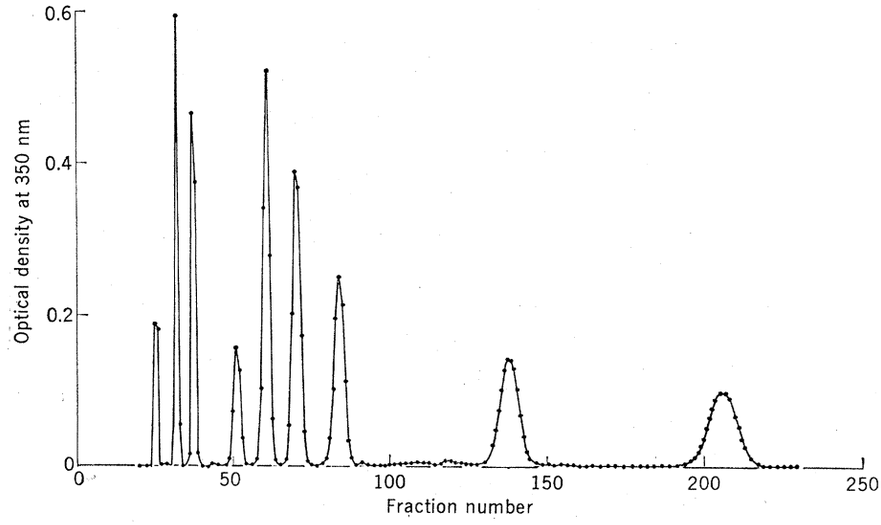

Fig. 2. Chromatogram for the separation of DNP amino acids. Peaks identified in order of elution and their partition coefficients from left to right are: *N*-dinitrophenyl-δ-L-ornithine (>100), *N*-dinitrophenyl-L-aspartic acid (3.8), *N*-dinitrophenyl-D,L-glutamic acid (1.9), *N,N'*-dinitrophenyl-L-cystine (0.94), *N*-dinitrophenyl-β-alanine (0.71), *N*-dinitrophenyl-L-alanine (0.56), *N*-dinitrophenyl-L-proline (0.45), *N*-dinitrophenyl-L-valine (0.26), and *N*-dinitrophenyl-L-leucine (0.18).

vides phase separation directed along a radius of rotation. The pressure necessary to move the moving phase is the sum of the differences in levels multiplied by the density difference and the *g* force (*2*). To overcome this pressure without a pressurized rotating seal, a syringe is mounted on the center of the rotating centrifuge head while the rotating plunger is pushed by a syringe driver through a thrust bearing.

To demonstrate the capability of the method we used a two-phase system of chloroform, glacial acetic acid, and 0.1*N* aqueous hydrochloric acid (2:2:1) (*3*) for the separation of dinitrophenyl (DNP) amino acids. Dinitrophenyl amino acids (Mann Research Laboratories, New York), which have suitable partition coefficients (Fig. 2), were dissolved in a portion of the lower phase with concentrations of 1 percent where the solubility permitted. The separation tube was filled with the stationary phase, and the sample solution (5 μl) was drawn into the entrance by gentle suction. Usually 2 to 3 μl of chloroform were also added to compensate for evaporation losses. The syringe was filled with the moving phase, and the necessary connections were made. Centrifugation was performed at room temperature at 900 to 950 rev/min with flow rates of 125, 200, 320, 510, and 820 μl/hour. Each fraction (eight drops) of eluate was mixed with 3 ml of 95 percent ethanol, and the optical density at 350 nm was determined with a Beckman DU spectrophotometer.

Figure 2 shows a typical separation for nine DNP amino acids obtained on a coiled column of 8000 turns and with a helix diameter of 0.85 mm prepared from a 40-m length of the Teflon tubing (0.2 mm, inner diameter) (Zeus Industrial Products, Princeton, N.J.) embedded in a transparent epoxy resin. The light phase was used as the moving phase at a flow rate of 125 μl/hour. The efficiency calculated from the formula used in gas chromatography (*4*) gave values ranging between 5200 and 2500 theoretical plates.

On the basis of our investigations we have estimated that the efficiency increases with tube length, smaller bore, and slower flow. Moreover, with a given length of tubing, we can increase efficiency by increasing the number of coil units *n*, and we can increase the number of coil units by decreasing the helix diameter. The significance of the first three factors is obvious by analogy with gas chromatography. However, the unique factor *n* merits further discussion. As illustrated in Fig. 1c, each coil unit shows an asymmetrical distribution of the two phases. One-half of the coil contains a longitudinal interface close to its inner surface whereas the other half is "dead space," that is, entirely occupied by the moving phase. In addition, a segment interface is present at the top and bottom of each coil. The length of the longitudinal interface is approximately half that of the uncoiled tube and is therefore independent of the number of turns. However, the number of segment interfaces is equal to twice the number of coil units. For a given length and bore of tubing, therefore, the total area of interface, and hence the efficiency, will increase with the number of turns. Furthermore, since the dead space and the effective space in each coil unit decreases in length with the helical diameter, longitudinal diffusion of the solute within these spaces will be reduced. If one phase is more viscous than the other, it is more desirable for column efficiency to have the less viscous phase as the stationary phase. The lower viscosity in the effective space of the stationary phase offers less resistance to diffusion of the solute and thus contributes to improved efficiency.

Clearly, then, for a given phase system, the efficiency of countercurrent chromatography may be improved by using greater lengths of more tightly wound, narrower bore tubing. The practical limit to this procedure is governed by the centrifugal field and the injection pressure necessary to promote interfacial flow. The minimum pressure *P* at the syringe plunger necessary to sustain flow may be calculated from Eq. 1

$$P = R\,\omega^2\,|\,\rho_s - \rho_m\,|\,n\,d \qquad (1)$$

where *R* denotes the distance between the center of rotation and the axis of the helix, ω is the angular velocity of rotation, *n* is the number of coil units, *d* is the helical diameter, and ρ_s and ρ_m are the densities of the stationary and moving phases, respectively (*5*). The pressure used during the separation of the DNP amino acids was 20 atm, which is close to the maximum attainable without causing severe distention or rupture of the Teflon tubing or leakage at the syringe plunger.

The resolving power is comparable to that of refined gas chromatographic methods, and yet the technique is more versatile in that nonvolatile materials may be resolved and the phase system used may be changed for programmed separations. Because no solid support is used, countercurrent chromatography offers a number of advantages over the conventional liquid-liquid partition chromatographic techniques. Tailing of the solute is obviated, the procedure is highly reproducible and free from contamination, and the separated solutes are readily recovered.

The applicability of the system to macromolecular samples, such as nucleic acids and polypeptides, has been confirmed on both conventional and

polymer phase systems. A preparative modification currently being explored has been successful in separating more than 100 mg of DNP amino acids.

YOICHIRO ITO
ROBERT L. BOWMAN
Laboratory of Technical Development, National Heart Institute, Bethesda, Maryland 20014

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2. We have observed that at low flow rates and with mobile solvents viscous resistance to flow is insignificant in comparison to opposing hydrostatic forces.
3. W. Hausmann, J. R. Weisiger, L. C. Craig, *J. Amer. Chem. Soc.* **77**, 723 (1955).
4. The efficiency was calculated from the formula

$$N = (4\ R/w)^2$$

where N is the number of the theoretical plates, R is the retention time referred to the peak maximum, and w is the peak width.
5. The validity of Eq. 1 has been confirmed by manometric measurement.
6. We thank Drs. G. G. Vurek and T. Tanimura for their assistance in experiments and Dr. D. H. Christopher for preparation of the manuscript. We are indebted to H. Chapman for fabricating the instrumentation and to Miss S. Kopit who participated as a summer student.

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Posttranscriptional Control in the Steroid-Mediated Induction of Hepatic Tyrosine Transaminase

Abstract. *The purine analog azaguanine does not inhibit the initial induction of hepatic tyrosine transaminase by hydrocortisone. However, the continued induced synthesis of tyrosine transaminase, elicited by repeated doses of hydrocortisone, is inhibited approximately 64 percent in the presence of the analog after 7 to 8 hours and appears to be almost completely inhibited by 9 to 10 hours; this suggests that the induction cycle involves the activation and renewal of a pool of preexisting messenger RNA.*

Because actinomycin D inhibits the corticosteroid-mediated inductions of hepatic tyrosine transaminase [L-tyrosine : 2-oxoglutarate aminotransferase (E.C. 2.6.1.5)] and tryptophan pyrrolase [L-tryptophan : oxygen oxidoreductase (E.C. 1.13.1.12)] (*1*), it has been inferred that the inductions require the synthesis and transport to the cytoplasm of newly formed messenger RNA (mRNA). However, actinomycin D is reported (*2*) to have a number of effects on RNA metabolism in addition to its inhibition of RNA synthesis. Therefore, we investigated the mechanism of induction of tyrosine transaminase with the purine analog 8-azaguanine, which inhibits protein biosynthesis as a result of its incorporation into mRNA (*3*).

Male Sprague-Dawley rats (250 g) were adrenalectomized 7 days before use and fasted 24 hours before the start of the experiment. 8-Azaguanine, dissolved as described previously (*3*), hydrocortisone sodium succinate (Solucortef, Upjohn), and 5-amino-4-imidazole-carboxamide (AIC) were administered intraperitoneally. Tyrosine transaminase and tryptophan pyrrolase activities were measured (*4*) in the supernatant fraction (105,000*g*) from the liver; one activity unit was defined in each case as the amount of enzyme catalyzing the formation of 1 μmole of product per hour at 25°C. Experiments designed to measure the rate of synthesis of tyrosine transaminase were performed according to standard immunochemical techniques (*5*, *6*) with minor modifications (*7*).

8-Azaguanine inhibits by 89 percent the initial induction by hydrocortisone of hepatic tryptophan pyrrolase, but not that of tyrosine transaminase; a similar differential effect of the nucleoside analog 5-azacytidine on the steroid-mediated inductions of these enzymes has also been reported (*8*). Since the induction of tryptophan pyrrolase is virtually completely inhibited, it is inferred that effective concentrations of 8-azaguanine nucleotides are present during the initial phases of the induction of tyrosine transaminase.

These results are consistent with the hypothesis that the hydrocortisone-mediated induction of tyrosine transaminase utilizes preexisting RNA. An alternative explanation is that the messenger for this enzyme can function normally even when a portion of its guanine residues are replaced by 8-azaguanine. The latter possibility was eliminated by the results of the experiment, shown in Fig. 1, which also provide information concerning the size of the preexisting pool of messenger RNA. Hydrocortisone was administered at 3-hour intervals to ensure the continued induction of tyrosine transaminase at its maximum rate in the control rats. The controls received AIC and saline, while the experimental rats received AIC and 8-azaguanine, with each dose of hydrocortisone. The AIC reduces the deamination of 8-azaguanine in rat liver (*9*) and thereby potentiates the inhibitory action of the analog (*3*); it also eliminates the superinduction of tyrosine transaminase which appears to depend on the extensive deamination of 8-azaguanine (*10*).

The continued induction of tyrosine transaminase is significantly decreased after about 6 hours in the presence of 8-azaguanine (Fig. 1). This approximately corresponds to the time at which maximum induction is attained after a single inducing dose of hydrocortisone. It appears, therefore, that after the initial stage of the induction an increasing proportion of the corticosteroid-induced synthesis of the enzyme is dependent on the synthesis of new mRNA which can be rendered defective by 8-azaguanine. That is, this messenger species does not function normally when it contains the base analog.

Table 1. Inhibition by 8-azaguanine of the continued induced synthesis of tyrosine transaminase elicited by repeated doses of hydrocortisone. Conditions identical to those in Fig. 1, except that the rats were killed either 3 or 8 hours after the initial dose of hydrocortisone. The rats received ^{3}H-leucine (New England Nuclear, 44 c/mmole) (80 μc/250 g) 20 minutes before removal of the liver. The radioactivity in hepatic tyrosine transaminase was estimated and corrected for differences between samples with respect to the specific radioactivity of the free leucine pool in the liver (*7*). The data represent the mean ± range for two rats. The rate of change in enzyme activity during the labeling period was estimated from the curves in Fig. 1 and is included for purposes of comparison (*11*).

Addition	Time (hours)	Enzyme radioactivity: Corrected per milliliter of liver extract (count/min)	Enzyme radioactivity: Relative to that of controls	Increase in enzyme activity during labeling (units per 100 mg of protein per hour)
		Saline control		
		140 + 4	1.0	
		Hydrocortisone + AIC		
Saline	3	508 ± 28	3.6	
8-Azaguanine	3	536 ± 24	3.8	
Saline	8	444 ± 44	3.2	35
8-Azaguanine	8	260 ± 20	1.8	20