Experiment No.	Amount of addition		Composition of solution		Amount of inoculation (g)	Resolved crystals[a]		
	DL-form (g)	Active form (g)	DL-form (g)	Active form (g)		Yield (g)	$[\alpha]_D^{25}$	Optical purity (%)
1	26.00	(L) 0.90	26.00	(L) 0.90	(L) 0.10	2.16	+ 7.50°	100
2	2.06	0	25.74	(D) 1.16	(D) 0.10	2.68	− 7.30°	97.3
3	2.58	0	25.55	(L) 1.35	(L) 0.10	2.43	+ 7.50°	100

[a] Specific rotation of pure L-alanine benzenesulphonate: $[\alpha]_D^{25} = +7.50°$ ($c = 2$, ethanol).

at the same temperature for 16 h. The precipitated crystals were filtered and 4.40 g of D-alanine benzene sulphonate was obtained. Anal. Found: *N*, 5.62%; calcd. for $C_9H_{13}O_5NS$: *N*, 5.66%. The product was optically pure, $[\alpha]_D^{25} = -7.50°$ ($c = 2$, ethanol). The D-alanine benzene sulphonate, 3.00 g, was dissolved in 60 ml of distilled water and passed through a column of Amberlite IR-120 in H-form. The D-alanine absorbed on the resin was eluted with 180 ml of 1*N*-NH_4OH and the elute was concentrated to dryness. The residue was crystallized from aqueous methanol to give 0.98 g of pure D-alanine (91% of the theoretical). Anal. Found: *N*, 15.70: calcd. for $C_3H_7O_2N$: *N*, 15.72. $[\alpha]_D^{25} = -14.60°$ ($c = 2$, 5*N* HCl).

For further optical resolution, the mother liquor can be used repeatedly to separate the other enantiomorph. Namely, the same amount of DL-modification as that of the enantiomorph previously separated out, is added to the mother liquor and dissolved at an elevated temperature. The supersaturated solution was cooled, seeded and crystallized in the same way as described above. By repeating these procedures, L- and D-alanine benzene sulphonates were successfully obtained. The examples of the first several runs in 100 ml scale are shown in the Table.

Benzene sulphonic acid in the effluent of ion exchangers charged with solution of optically active alanine benzene sulphonate was readily recovered as DL-alanine benzene sulphonate by the addition of the corresponding amount of DL-alanine to the effluent and by further concentration of the solution.

Thus the total optical resolution of DL-alanine benzene sulphonate can be accomplished. This simple procedure is considered to be one of the most advantageous methods for optical resolution of DL-alanine, because the method requires neither optically active resolving agent nor conversion of DL-alanine into complicated derivatives.

Zusammenfassung. Die direkte optische Spaltung von DL-Alaninbenzensulfonat in die optischen Antipoden wurde bewirkt durch die bevorzugte Kristallisation aus übersättigter Lösung, die mit einem der reinen optisch-aktiven Kristalle inokuliert war.

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A Simple Accurate Method for Determining the Activity of Proteolytic Enzymes

We developed a simple modified method for the determination of free amino acids by means of ninhydrin[1], making use of a Summerson manometer and two-compartment flasks (Figure 1), widely used in conventional manometric techniques[2].

The analysis is performed as follows: 1 ml of the solution to be tested is placed in the main compartment D (Figure 1). In the corresponding sidearm C are placed 0.5 ml of 0.1% ninhydrin solution in 0.1*M* citrate buffer pH 4.7. The other compartment E contains 1 ml of 0.5*N* NaOH and the corresponding sidearm F 0.5 ml of 3*N* lactic acid. Another flask is similarly equipped, with the main compartment containing 1 ml of the citrate buffer (control flask). The 2 flasks are connected with the Summerson manometer A and allowed to thermostate in a water bath at 75 °C for 10 min and shaken at 100 cpm. The flasks are then closed by turning the three-way stopcock and the ninhydrin is added to the amino acid solution. After 10 min, the water bath temperature is lowered to 30 °C and the flasks are opened towards the exterior excluding the manometer arms, to equilibrate again the internal pressure. After 10 min the flasks are connected with the manometer arms and the lactic acid is added to the NaOH. The developed CO_2 quantities are calculated at the end of the variations of manometric pressure. The CO_2 coming from the decarboxylation of the free or terminal amino acid is calculated by subtracting, from the amount of CO_2 developed in the flask containing the amino acid, the amount of CO_2 developed in the flask without amino acid.

The method is useful for substances developing 1 to 10 μmoles of CO_2. The method gives accurate results even using non-CO_2-free reagents, as it results from Table I.

[1] D. D. VAN SLYKE, D. T. DILLON, MCFADYEN and P. HAMILTON, J. biol. Chem. *141*, 627 (1941).

[2] W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, *Manometric Techniques* (Burgess Publishing Co., Minneapolis 1959).

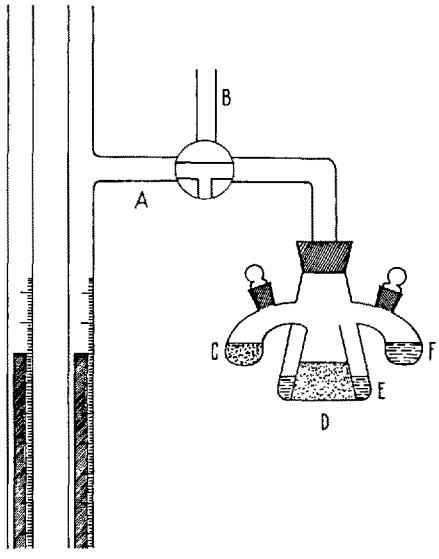

Fig. 1. Diagram illustrating the manometric apparatus.

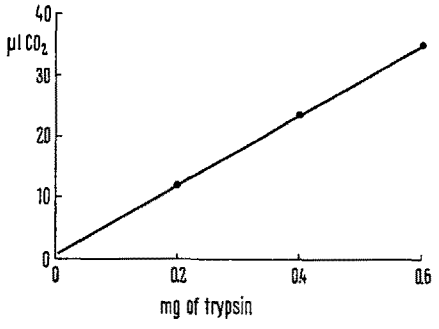

Fig. 2. Digestion of casein (2 mg/ml) by increasing amounts of trypsin in 0.1 *M* phosphate buffer pH 7.8 at 35 °C for 2 h.

Table I. CO_2 from decarboxylation of amino acids by ninhydrin

Amino acid	μl CO_2/μmoles amino acid	CO_2 moles evolved moles amino acid
Alanine	21.4 ± 0.60[a]	1
Asparaginic acid	45.1 ± 1.30	2
Cystine	21.6 ± 0.50	1
Glycine	23.5 ± 0.75	1
Methionine	21.9 ± 0.40	1
Phenylalanine	20.8 ± 4.00	1
Proline	23.2 ± 1.70	1
Tryptophane	21.3 ± 1.03	1
Tyrosine	20.4 ± 1.80	1

[a] Standard deviation.

Table II. Degradation of natural and synthetic peptides

Enzyme	Substrate	Degradation CO_2 μmoles/substrate μmoles
Cathepsin C	Glycilphenylalaninammide	0.80
	Poly-D,L-glycine DP = 76	not digested
	Poly-L-leucine DP = 8.5	not digested
Renin	Poly-L-leucine DP = 8.5	10.5
	Poly-L-proline DP = 14.5	not digested
	Poly-D,L-glycine DP = 76	not digested
Trypsin	Poly-D,L-tyrosine DP = 4.5	5.2
	Poly-L-proline DP = 14.5	not digested
	Poly-L-leucine DP = 8.5	not digested
	Casein (M.W. 121800)	102

Conditions: cathepsin C: incubation in 0.1 *M* citrate buffer pH 6.0 at 39 °C for 20 min; renin: incubation in 0.15 *M* phosphate-saline buffer pH 5.7 at 37 °C for 15 h; trypsin: incubation in 0.1 *M* phosphate buffer pH 7.8 at 35 °C for 2 h.

Owing to the fact that peptide hydrolases set free amino acid-COOH, their possible decarboxylation with ninhydrin is a function of the peptides degradation.

Figure 2 and Table II show results obtained with natural and synthetic polypeptides with cathepsin C, renin and trypsin.

The control flask contains all the reagents incubated with heat-inactivated enzymes. With particular regard to the cathepsin C, at pH 6.0 the enzyme can have some polymeric activity[3,4].

Riassunto. Nel presente lavoro é descritta una semplice e rapida metodica, eseguita con i convenzionali metodi manometrici, per dosare l'attività proteolitica di enzimi.

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[3] R. M. METRIONE, A. G. NEVES and I. S. FRUTON, Biochemistry *5*, 1597 (1966).

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