CHROMATOGRAPHIC SEPARATION OF DIASTEREOISOMERIC ESTERS

I. LACTATE AND MANDELATE OF BUTANOL-2*

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The resolution of racemic alcohols is generally a more difficult task than the resolution of acids or amines. The classical method for alcohols, which is still widely used, consists in converting the alcohol to the acid ester of a diprotic acid and then applying fractional crystallization to the salt of this derivative with an optically active amine. Several workers have studied the separation of diastereoisomeric esters by gas–liquid[1–4], liquid–liquid[5], and solid–liquid[6,7] chromatography; but none of them has developed a method that is conspicuously better than the classical.

The purpose of this paper is to describe the resolution of racemic alcohols by chromatography of their diastereoisomers with an ordinary, optically inactive, ion-exchange resin as the stationary phase and aqueous solutions of salts or nonelectrolytes as the mobile phase.

EXPERIMENTAL

Synthesis of the diastereoisomeric esters

The mandelic acids were obtained from Aldrich Chemical Company. The D(—)-mandelic acid had $[\alpha]_D^{23.5} = -154.1$ ($C = 2.077$ in H_2O) and $[\alpha]_D^{21.0} = -153.9$ ($C = 2.917$ in H_2O). The menthol, lactic acid, and butanol-2 were obtained from Eastman, Mallinckrodt, and Matheson, Coleman and Bell respectively.

Equal weights of the alcohol and acid were mixed with about 0.6 ml of concentrated sulfuric acid (hydrochloric acid in the case of menthyl mandelate) and 75 ml of benzene. The mixture was refluxed on a water bath until no more water was obtained in a Dean-Stark tube. It was then dissolved in ether and washed with water, aqueous sodium carbonate, and again with water until it was neutral. It was dried with anhydrous sodium sulfate and filtered. After distillation of the ether and benzene, the residual ester was purified by distillation at low pressure. Yields were between 50 and 78 %. The DL-*sec.*-butyl D(—)-mandelate melted at 26–27°; $[\alpha]_D^{21.0} = -96.6$ ($C = 0.969$ in EtOH). The DL-*sec.*-butyl DL-lactate boiled between 57.0 and 58.5° at 8 mm.

Chromatography

The dimensions of the several columns will be given with the results. The 11.3-m column was made from five lengths of glass tubing. Neoprene gaskets were inserted between the flanged ends and clamped together. This column was located in a shaft

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that extended from the basement to the third floor of Wright Laboratory. Although automatic temperature control was not available, the internal brick walls of the shaft provided a much more nearly constant temperature than prevails in an ordinary laboratory. All columns were vertical, and the mobile phases moved downward at flow rates controlled by a Minipump*. Fractions of effluent were generally collected automatically. Glass tubing was used as much as possible to conduct the liquid from the reservoir through the pump and column to the fraction collector. Where necessary, glass-to-glass joints were connected with Tygon tubing. These precautions decreased the dissolution of ultraviolet-absorbing compounds from plastic or rubber tubing[8]. In the case of butyl lactate, the effluent passed into a flow-through cell contained in a Beckman DB spectrophotometer; and the absorbance was recorded automatically. The Dowex resins used in the columns had been treated by standard procedures to remove the excessively fine particles and—as much as practicable—the soluble organic constituents.

Solubility measurements

The solubility of the esters was determined by shaking the appropriate solvent (water or aqueous solutions of ethanol or sodium chloride) with excess of the ester for 24 h, separating the two phases, and measuring the absorbance of the aqueous phase at 257.5 mμ. Although the accuracy of these measurements is not very great, it is sufficient for the present purpose.

A Beckman DU spectrophotometer and a Rudolph Model 80 polarimeter were used in the examination of the fractions.

RESULTS AND DISCUSSION

D(—)-*Menthyl* DL-*mandelate*

The solubility of this compound in water is so small that aqueous ethanol (35 to 50 vol. %) was used as the eluent in elution chromatography. Then the peak of the graph appeared shortly after the interstitial volume, and no separation could be observed. The addition of lithium or sodium chloride to the eluent did not help. The substitution of dioxane for ethanol was also fruitless.

The unsuccessful attempt to separate these diastereoisomers may be attributed to the following factors:

(1) Concentrations of organic solvents necessary to give sufficient solubility to the ester cause very small separation factors.

(2) Solubilization chromatography has been found to be useful only for compounds of about 12 or fewer carbon atoms .[9]

(3) Entrance of the ester into the resin is inhibited because of its small solubility and large molecular volume. The steric effects caused by the ester are due to the bulky groups on the menthol and its nonplanar nature. (Menthol is predominantly in the chair form.)

DL-*sec.-Butyl* DL-*mandelate*

The solubility of this ester in water and some aqueous solutions is given in Table I.

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Four ml of nearly saturated aqueous solution was eluted with pure water through a column 150.5 cm × 0.785 cm^2 of Dowex 50W-X2, 200–400 mesh, sodium form, at 0.32 cm per min. Fractions of 3.60 ml were analyzed spectrophotometrically at 257.5 mμ. The graph showed two very distinct but badly overlapping peaks. It is clear that the original mixture of four diastereoisomers had been partly separated into a mixture of the D–L and the L–D esters and a mixture of the L–L and the D–D esters. It could not be ascertained from the graph which mixture constituted each peak. The distribution ratios of the two pairs of diastereoisomers were calculated[10] from the retention volumes to be $C_1 = 11.2$, $C_2 = 12.1$.

TABLE I

SOLUBILITY OF DL-*sec.*-BUTYL ESTERS AT ROOM TEMPERATURE

Solvent	*Solubility (moles per l)*	
	DL-*Mandelate*	DL-*Lactate*
Water	0.015	0.505
10% aqueous EtOH (v/v)	0.023	
20% aqueous EtOH (v/v)	0.035	
30% aqueous EtOH (v/v)	0.068	
0.10*M* NaCl	0.015	
0.50*M* NaCl	0.013	0.400
1.0*M* NaCl	0.012	
1.5*M* NaCl		0.250
2.0*M* NaCl		0.222
3.0*M* NaCl		0.130

In the hope of getting a better separation, the elution was repeated with 0.50*M* and 1.0*M* aqueous sodium chloride as eluent[11]. In the former case, the values of C_1 and C_2 were 17.6 and 19.4. The small increase in the ratio C_2/C_1 did not compensate for the additional time required for the elution because of the increase in C values. Therefore salting-out chromatography of this ester was abandoned. An elution was done under similar conditions except that the eluent was a 15 vol.% solution of ethanol in water to take advantage of the greater solubility of the ester in this solvent. The separation was less complete than with pure water as eluent; hence work with solubilization chromatography[12] was discontinued.

DL-*sec.*-*Butyl* D(—)-*mandelate*

Five ml of a saturated solution of this ester in pure water (containing 0.074 mmole) was eluted through a column 151.6 cm × 0.785 cm^2 of sodium-form Dowex 50W-X2, 200–400 mesh, at 0.65 cm per min with pure water as eluent. Repetition of the foregoing experiment at a flow rate of 0.32 cm per min significantly improved the separation. The elution graph of the latter experiment, Fig. 1, is very similar to that of the elution of DL-*sec.*-butyl DL-mandelate with water. By the application of previously published equations[10] to this graph, the following values were calculated: $C_1 = 10.8$, $C_2 = 11.6$, P_1 (calculated from the rising slope of the first peak) = 9.7, P_2 (calculated from the falling slope of the second peak) = 7.8, H = the height of column required for a chromatographic separation 99.9% complete = 1000 cm.

The elution was repeated under identical conditions except that the resin was

in the barium form, but no advantage was found. The hydrogen form would catalyze the hydrolysis of the ester. All subsequent work was done with the sodium form.

Elutions of DL-*sec.-butyl* D(—)-*mandelate through the long column.* Fifty ml of a saturated aqueous solution of this ester was added to a column 10.1 m × 1.29 cm^2 of sodium-form Dowex 50W-X2, 200–400 mesh. This was eluted with water at a flow rate of 0.26 cm per min. Fractions of 7.73 ml were collected and examined as before. The elution graph, Fig. 2, shows a nearly quantitative separation of the two diastereoisomers.

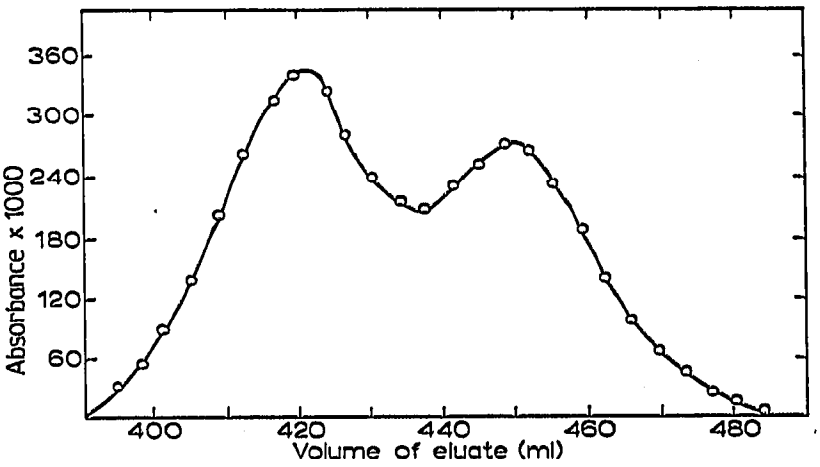

Fig. 1. Elution graph of DL-*sec.*-butyl D(—)-mandelate with a 152-cm column.

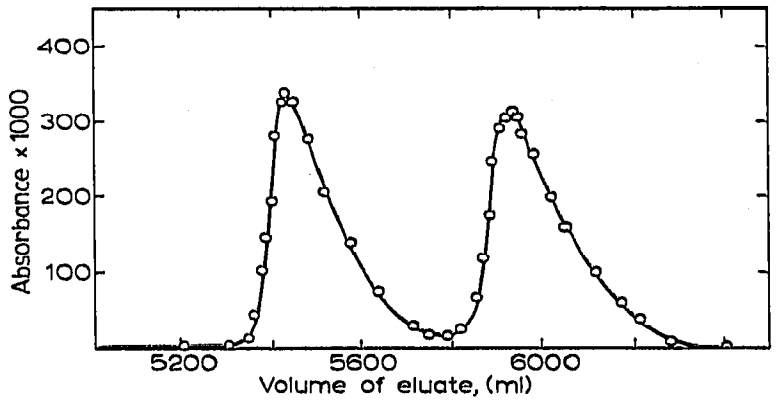

Fig. 2. Elution graph of DL-*sec.*-butyl D(—)-mandelate with a 10-m column.

In spite of elaborate precautions to wash soluble organic impurities from the resin before use, troublesome absorbances were observed in the fractions of eluate before the breakthrough of the first diastereoisomer and after the complete elution of the second. These absorbances were greater after than before the elution. Fig. 2 was drawn after correcting for the blanks by assuming that the blank increased linearly from a point just before the breakthrough to a point just after the complete elution.

Both elution curves of Fig. 2 show distinct tailing. This is probably due in part to the variation in the blank correction, which skews the graphs so as to aggravate tailing. Failure to attain equilibrium within the column would also contribute to the tailing.

For equal concentrations of esters, the fractions under the first curve had more

positive rotations than those under the second. This indicates that L(+)-*sec.*-butyl D(—)-mandelate was eluted before the D(—)-*sec.*-butyl ester. The areas under the two curves had a ratio of 1.01, indicating that the sample contained the two diastereoisomers in equal amounts within the experimental error.

Ultraviolet scans of fractions from each peak coincided with those of the pure diastereoisomers when the effluent (before the solute emerges) was used as the blank.

The values of C_1 and C_2 calculated from Fig. 2 are 12.75 and 14.02. A repetition of this elution gave 12.71 and 13.98. These results are in very good agreement with each other. The failure to agree well with the results from the shorter columns is due to the fact that a different batch of Dowex 50W-X2 was used to fill the long column.

Frontal chromatography of DL-*sec.-butyl* D(—)-*mandelate on the long column.* In spite of the nearly quantitative separation of the diastereoisomers achieved by elution chromatography on the long column, this procedure is not a practicable method of resolving racemic butanol-2 because of the very severe limitation on the size of the sample, 0.037 mmole of each diastereoisomeric ester with the column used. Larger samples can be accommodated by frontal chromatography.

A nearly saturated aqueous solution of DL-*sec.*-butyl D(—)-mandelate (0.0074*M* with respect to each diastereoisomer) was fed to the 10.1-m column at 0.24 cm per min until 5,710 ml of effluent was collected. Then the ester solution in the tube above the resin was removed and water was fed into the column at the same rate until both diastereoisomers were removed from the column. Fractions of 10.0 ml of effluent were collected.

In order to eliminate the large blank readings of absorbance at 257.5 mμ, the concentrations of ester in the fractions were determined by a slight modification of the spectrophotometric dichromate method[13]. Instead of the recommended volumes, 4 ml of sample solution and 14 ml of water were added to 18 ml of the 0.1*N* dichromate in concentrated sulphuric acid. Since water dissolves from the resin only very small concentrations of solutes having extremely large absorptivities in the ultraviolet, the use of the dichromate method eliminated the blank correction almost entirely.

Fig. 3 shows the chromatographic curve for the entire cycle, *i.e.*, the frontal and the subsequent elution of the esters from the resin. The first isomer, L(+)-*sec.*-butyl D(—)-mandelate, appeared in the effluent at U (volume of effluent) = 5,260. Its concentration rose rapidly to 0.0081*M*. Then it remained essentially constant. The second diastereoisomer appeared in the effluent at U = 5,710. The total concentration

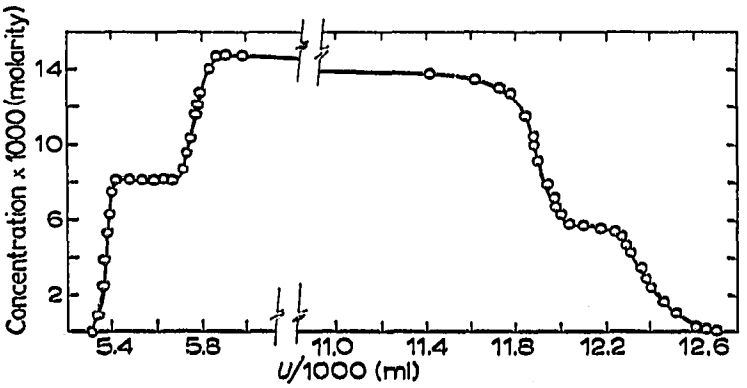

Fig. 3. Frontal graph of DL-*sec.*-butyl D(—)-mandelate with a 10-m column.

of ester rose rapidly and reached a plateau at 0.00147 M at $U = 5{,}870$. The concentration of the first diastereoisomer started to decrease at $U = 11{,}420$ and became zero at $U = 12{,}100$. At this point the second ester was emerging from the column with a concentration of about 0.0057 M. Its concentration started to decrease at $U = 12{,}180$ and reached zero at $U = 12{,}710$.

The theory of frontal partition chromatography with ion-exchange resins as the stationary phase has been discussed, and data were presented that support the theory very well[14]. Application of the equations of this paper to the data of Fig. 3 permits the calculation of the C values of each ester from the midpoints of their breakthroughs. The values are 12.55 and 13.50 respectively. The distribution ratios can also be calculated by the equation

$$U_d^* - U_e = CV + V \qquad (1)$$

where U_e is the volume of effluent collected when the input into the column was changed to water and U_d^* is the volume of effluent at the midpoint of the descending part of the graph for any one solute. This equation gave values of 12.35 and 13.67, respectively, in fair agreement with those obtained from the first part of this experiment and from the elutions performed with the same batch of resin.

Fig. 3 has some unexpected features: (1) The first plateau, corresponding to the emergence of pure L(+)-*sec.*-butyl D(—)-mandelate from the column, occurred at a concentration slightly greater than the concentration of D(—)-*sec.*-butyl D(—)-mandelate when it finally reached the second plateau. (2) The second plateau had a small negative slope; and the third plateau, corresponding to the removal of pure D(—)-*sec.*-butyl D(—)-mandelate from the column, occurred at an apparent concentration lower than that calculated from the difference between the second and first plateaus. (3) The portion of the graph corresponding to the emergence of pure L(+)-*sec.*-butyl ester ($U = 5{,}300$ to $5{,}700$) is more nearly rectangular than the portion corresponding to the emergence of pure D(—)-*sec.*-butyl ester ($U = 12{,}000$ to $12{,}700$).

The area under the graph from $U = 5{,}300$ to $U = 5{,}700$ represents 2.6 mmole of very nearly pure D(—)-*sec.*-butyl D(—)-mandelate. The area from $U = 12{,}000$ to $U = 12{,}700$ represents approximately the same quantity (2.3 mmole) of very nearly pure L(+)-*sec.*-butyl mandelate.

The fractions of effluent from $U = 5{,}300$ to $U = 5{,}700$ that had not been destroyed in the determinations of concentration were pooled. The ester was extracted by ether and hydrolyzed by aqueous sodium hydroxide. The L(+)-butanol-2 was extracted by ether. The rotation of this solution was +0.207°. An ethereal solution of butanol-2 recovered analogously from the effluent from $U = 12{,}000$ to U 12,700 was —0.156°. These data indicate that a resolution of the butanol had been accomplished. Unfortunately, these rotations cannot be used to check the yields of diastereoisomers previously mentioned (2.6 and 2.3 mmole) because the authors were unable to find in the literature a value of $[\alpha]_D$ of butanol-2 in ether and because extensive loss by evaporation occurred during the saponification.

Repetitive frontal chromatography of DL-*sec.-butyl* D(—)-*mandelate on the long column.* A chromatographic experiment was performed like the last except that the sequence of feed to the column was 720 ml of aqueous ester solution, 2095 ml of water, 700 ml of ester solution, 2100 ml of water, 680 ml of ester solution, and finally sufficient

water to remove all the esters from the column. It is suggested that this type of separation be called *repetitive frontal chromatography*.

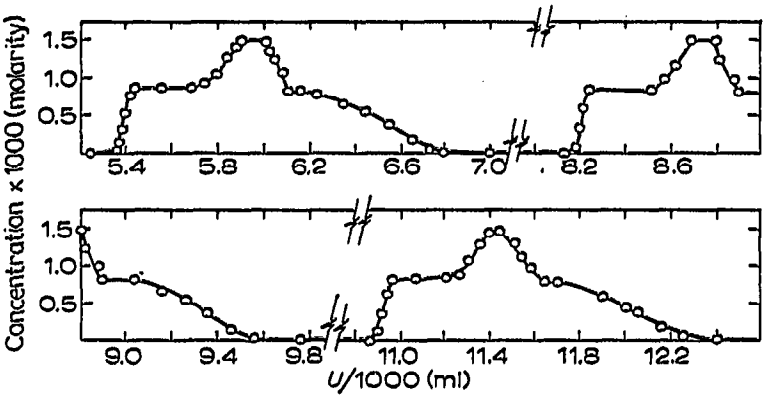

Fig. 4. Graph of repetitive chromatography of DL-*sec.*-butyl D(—)-mandelate with a 10-m column.

A comparison of Figs. 3 and 4 reveals the advantage of this procedure. Fig. 4 has three regions representing pure L(+)-*sec.*-butyl D(—)-mandelate, each approximately equal in area to the single analogous region of Fig. 3. The same statement is applicable to the D(—)-*sec.*-butyl ester. Six independent values of C of the L(+)-*sec.*-butyl ester and six of the D(—)-*sec.*-butyl ester can be calculated from the data of Fig. 4. The mean results are 12.41 and 13.53 with standard deviations of 0.14 and 0.10, respectively, in satisfactory agreement with the values obtained in the previously discussed frontal and elution experiments. On the other hand, the calculated[12] values of P, the number of plates per cm of column, are in poor agreement among themselves and with the values obtained in elution chromatography. This may be due to the fall in concentration of the L(+)-isomer emerging from the column when the D(—)-isomer starts to emerge. This would make the second plateau lower than the theoretical value, cause small errors in the C values estimated from the midpoint between the first and second plateaus, also from the midpoint between the second and third plateaus, and make any calculations of P evaluated in these regions highly dubious.

From the foregoing experiment, results show that 360 ml of frontal solution was required to reach the end of the first plateau. Therefore, the addition of the frontal-feed solution beyond this volume is actually unnecessary as the second plateau represents a mixture of the two diastereoisomers. One could have also reduced the volume of eluent between the end of the "elution" and the next frontal breakthrough by at least one liter. If these modifications were made, then one could add seven frontal samples instead of three within a total volume of 11,800 ml. This would have more than doubled the amounts of the optically active alcohols that could have been recovered. In comparison to the initial frontal-elution experiment, the total amounts of optically active isomer would have been seven times as great. This technique could be applied to other two-component systems.

There are two features that militate against the convenience of this method of resolving racemic alcohols: (1) The small value of C_2/C_1 together with the large values of these constants requires the use of an inconveniently long column. (2) The small solubility of the mandelate esters requires the use of dilute feed solutions and hence

involves the recovery of the active alcohols from dilute solutions of their esters. The substitution of lactate esters for mandelate esters should permit the use of more concentrated feed solutions.

DL-*sec.*-*Butyl* DL-*lactate*

The much greater solubility of this ester in comparison with the mandelate (Table I) is an important advantage because it permits the use of more concentrated solutions.

Samples of this ester were eluted through columns, about 150 cm × 0.785 cm^2, of sodium-form Dowex 50W-X2, 200–400 mesh, with water and several different concentrations of sodium chloride as eluents at flow rates of 0.30 cm per min. Water gave a single broad peak. 0.5 *M* sodium chloride gave two barely distinguishable peaks. More concentrated aqueous solutions of sodium chloride gave two distinct but badly overlapping peaks. This indicates a partial separation of the four diastereoisomers into one mixture containing principally D-*sec.*-butyl D-lactate and L-*sec.*-butyl L-lactate and another mixture containing mostly D-*sec.*-butyl L-lactate and L-*sec.*-butyl D-lactate. It also indicates that racemic butanol-2 can be resolved by the chromatographic separation of its esters with active lactic acid.

The best eluent for the separation seemed to be 1.5 *M* aqueous sodium chloride. From the elution graph, Fig. 5, it was calculated[10] that a column of 11 m will give a quantitative separation.

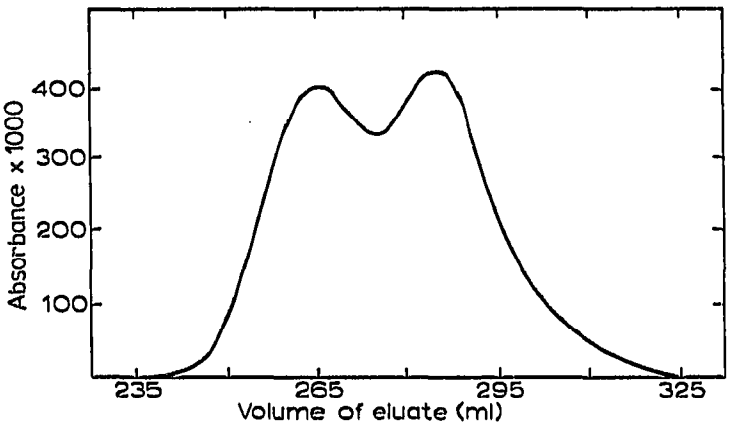

Fig. 5. Elution graph of 1.50 mmole of DL-*sec.*-butyl DL-lactate with a 150-cm column.

Additional details on the work described in this paper can be found elsewhere[15].

Work on the resolution of racemic alcohols by the chromatographic separation of their D-lactates is continuing.

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SUMMARY

A chromatographic procedure is described in which aqueous solutions of DL-*sec.*-butyl D(—)-mandelate and pure water were fed alternately to a column, 10 m × 1.29 cm², of Dowex 50W-X2. The time required for the chromatography was 680 h. About 7 mmole of D-*sec.*-butyl D-mandelate (contained in 1.1 l of water) and an equal amount of L-*sec.*-butyl D-mandelate (contained in 2.4 l of water) were isolated. Evidence is presented that much larger yields of the active *sec.*-butyl D-lactates can be obtained under approximately the same conditions because of the much greater solubility of the lactates.

REFERENCES

1 E. GIL-AV, R. CHARLES-SIGLER, G. FISCHER AND D. NUROK, *J. Gas Chromatog.*, 4 (1966) 51.
2 H. C. ROSE, R. L. STERN AND B. L. KARGER, *Anal. Chem.*, 38 (1966) 51.
3 J. CASANOVA AND E. J. COREY, *Chem. Ind. (London)*, (1961) 1664.
4 G. E. POLLOCK, V. OYAMA AND R. D. JOHNSTON, *J. Gas Chromatog.*, 3 (1965) 174.
5 S. BLACKBURN AND P. TETLY, *Biochim. Biophys. Acta*, 20 (1956) 423.
6 R. CURTI AND V. COLOMBO, *J. Am. Chem. Soc.*, 74 (1952) 3961.
7 M. M. JAMISON AND E. E. TURNER, *J. Chem. Soc.*, (1942) 611.
8 J. S. FRITZ AND G. E. HEDRICK, *Anal. Chem.*, 37 (1965) 1015.
9 W. RIEMAN, *J. Chem. Educ.*, 38 (1961) 338.
10 J. BEUKENKAMP, W. RIEMAN AND S. LINDENBAUM, *Anal. Chem.*, 26 (1954) 505.
11 R. SARGENT AND W. RIEMAN, *J. Phys. Chem.*, 61 (1957) 354.
12 J. SHERMA AND W. RIEMAN, *Anal. Chim. Acta*, 18 (1958) 214.
13 R. SARGENT AND W. RIEMAN, *Anal. Chim. Acta*, 14 (1956) 381.
14 H. D. SPITZ, H. L. ROTHBART AND W. RIEMAN, *Talanta*, 12 (1965) 395.
15 H. D. SPITZ, *Thesis*, Rutgers, The State University, New Brunswick, N.J., 1966.