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# PREPARATIVE GAS CHROMATOGRAPHIC RESOLUTION OF 3,3-DIMETHYL-2-BUTANOL AND OTHER ALCOHOLS*,**

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SUMMARY

The feasibility of preparative gas chromatographic resolution of small quantities of certain alcohols as the N-trifluoroacetyl-L-alanyl diastereomeric esters was demonstrated. The degree of resolution attained and the quantity resolved were strongly affected by the column length. Utilizing a 1 × 1584 cm column, both optical forms of 3,3-dimethyl-2-butanol were obtained with greater than 98 % purity.

## INTRODUCTION

In instances where only small amounts (*ca.* 1 ml or less) of both optically active forms of a particular alcohol are required, preparative gas chromatographic resolution would offer certain advantages over the classical method of alcohol resolution[1]. Small amounts of the resolved alcohol could easily be obtained without waste and without the necessity of working with toxic alkaloids. In theory, both optical forms could be obtained in one operation rather than through numerous recrystallizations and the change of the alkaloid base often involved in obtaining both optical forms of a particular alcohol. The optical purity could also be immediately and conveniently determined by analytical gas chromatography.

Recently, correlative to the gas chromatographic resolution of amino acids, the possibility of resolving alcohols by gas chromatography has been demonstrated[2]. Such work has been done predominately towards analytical goals, much of it with long capillary columns.

Motivated by the need for small amounts of both optical isomers of 3,3-dimethyl-2-butanol, the following study was initiated to investigate the possibility of resolving this alcohol, as well as others, by preparative gas chromatography.

## MATERIALS AND METHODS

### *Instruments and equipment*

The instrument used in this study was a Model 600 Series Research Specialties gas chromatograph equipped with a dual hydrogen flame detector. The preparative

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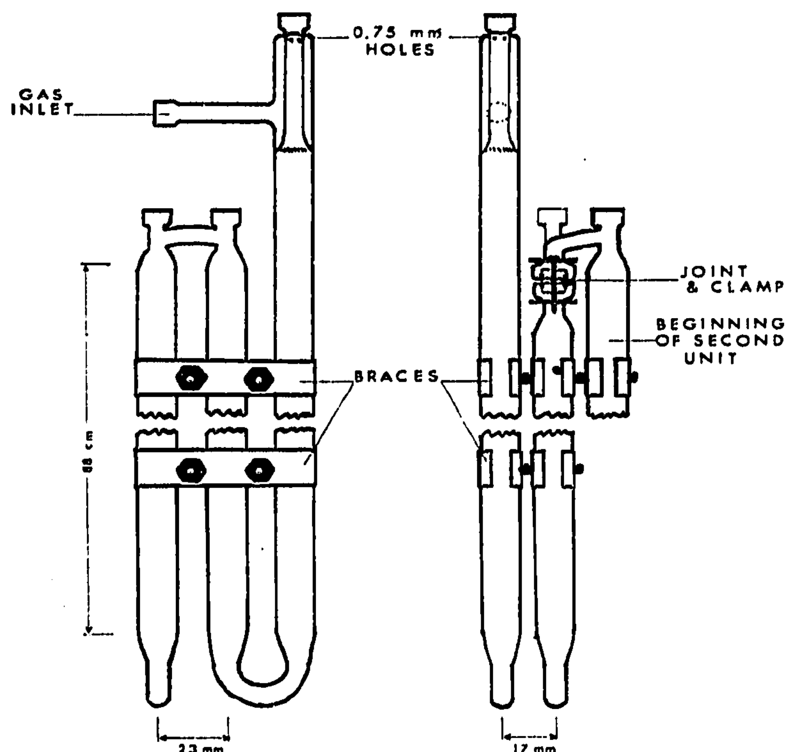


Fig. 1. Diagram of first unit and first section of the second unit of the preparative GLC column used to resolve the various diastereomeric esters.

column (Fig. 1) was constructed of three main units. Each unit consisted of six vertical sections of 10 mm I.D. pyrex tubing *ca.* 88 cm long connected together by 4 mm I.D. horizontal crossovers. Septum sockets were mounted directly above the vertical columns on the top of the crossovers. An all glass condenser type injector head, constructed so the carrier gas would enter the column through four 0.75-mm holes just beneath the injection septum was attached to the initial vertical column of the first unit. The three main units could be connected by means of 5-mm Fischer Porter Solv Seal® joints (Fischer Porter Co., Warminster, Pa., U.S.A.), which were held tightly together by means of a clamp consisting of steel collars (tapped and fitted with two small machine screws) on each joint socket. Thus the column could be operated at three lengths, *ca.* 528 cm, 1056 cm or 1584 cm.

The column was filled through the septum holders and compacted with a vibrator. A 1/8–1/4 in. plug of silanized glass wool was lightly compacted into the junctures of the crossovers and vertical columns.

A splitter consisting of a stopcock and a septum socket (inserted between the terminus of the column and the stopcock) containing a septum pierced by a piece of 1/16 in. stainless steel tubing which led to the hydrogen flame detector was installed in the all glass outlet. The split ratio was coarsely adjusted by the stopcock while fine adjustments were made by adjusting the hydrogen line pressure to the detector. The outlet tube was heated by means of an electrical heating tape.

Two 11 × 1 cm vacuum traps modified with $\mathfrak{S}$ balls to fit the column outlet and cooled with a dry ice–acetone bath were used to collect the material from the column.

*Derivative preparation*

*±3,3-Dimethyl-2-butyl-N-trifluoroacetyl-L-alanine.* A mixture of 50 ml of ±3,3-dimethyl-2-butanol containing 1.2 mequiv. of HCl per ml was reacted with 1 g L-alanine at 100° for 1 h in an oil bath-magnetic stirrer. The more volatile components of the mixture were removed from the alanine and alanyl ester by fractional distillation and the alcohol fraction (118–122°) saved and measured volumetrically. An additional 1.2 mequiv. of HCl per ml was added to the alcohol and the mixture was again reacted with 1 g of L-alanine as before. This procedure was continued until four 1-g quantities of alanine had been used. The combined alanine–alanyl ester residues were partitioned between about equal volumes of ether and 1 *M* $Na_2CO_3$. The carbonate fraction was extracted quantitatively with ether; the ether was removed on a rotating evaporator and the residue dissolved in 10–15 ml methylene chloride. This was reacted at room temperature with about a 1.5 *M* excess of trifluoroacetic anhydride. The more volatile components were removed by a rotary evaporator at 60° and the remaining residue vacuum distilled. A distinct non-viscous fore-run occurred followed by the very pale yellow more viscous N-trifluoroacetyl alanyl ester. In this manner, 10.4 g of the N-trifluoroacetyl alanyl ester was obtained.

*±2-Pentyl-N-trifluoroacetyl-L-alanine.* 2-Pentyl-N-trifluoroacetyl-L-alanine was prepared in a manner similar to the preparation of the corresponding 3,3-dimethyl-2-butyl derivative except that more amino acid and alcohol–HCl mixture were used initially and only one esterification was carried out.

*Other amino acid derivatives.* Small amounts of ±3,3-dimethyl-2-butyl esters of valine, phenylalanine and proline were prepared in a manner similar to the preparation of the corresponding alanyl ester. Smaller quantities of alcohol and amino acid were used and only one esterification and no vacuum distillation was carried out. These esters along with small amounts of the corresponding alanyl ester were converted to their N-alkyl derivatives by means of acetic anhydride, trifluoroacetic anhydride, propionyl chloride or trichloroacetyl chloride.

Small quantities of 1,1,1-trifluoro-2-propyl-N-trifluoroacetyl-L-alanine were also prepared in a similar manner.

*Effect of liquid phase and particle size*

To test the suitability of various liquid phases for resolving ±3,3-dimethyl-2-butanol the following liquid phases were tested in 4 mm I.D. analytical columns: cyclohexanedimethanol adipate, tetramethylcyclobutanediol adipate, 1,2,3-tris(2-cyanoethoxy)propane, OV-1 (dimethyl silicone), OV-17 (phenyl methyl silicone) and OV-25 (phenyl methyl silicone). To test the suitability of various diastereomers for the resolution of 3,3-dimethyl-2-butanol, the N-acetyl, N-trifluoroacetyl, N-trichloroacetyl and N-propyl derivatives of the alanyl, valyl, phenylalanyl and prolyl esters of the alcohol were chromatographed on these columns. The N-trifluoroacetyl ester chromatographed on either the OV-1 or OV-17 columns appeared to give the most favorable results. Columns (1 × 528 cm), packed with either 45/60 or 100/120 mesh DMCS-treated acid-washed Chromosorb W® coated with either 10 % OV-1 or OV-17 were used to determine the effect of particle size and of the two liquid phases on preparative scale separations. The coarser packing appeared to have a slightly higher overload capacity while OV-1 appeared to give resolutions slightly superior to those given by OV-17.

In order to appraise the effect of column length on the quality of separation, the three sections of the preparative column were packed with 10% OV-1 on 45/60 DMCS-treated acid-washed Chromosorb W. The column was operated at 165° with a 230 ml/min nitrogen flow rate. Injections of 5, 10, 15, and 20 $\mu$l of $\pm$3,3-dimethyl-2-butyl-N-trifluoroacetyl-L-alanine were made into the column. This procedure was then repeated for the two shorter lengths.

*Resolution of the $\pm$3,3-dimethyl-2-butyl-N-trifluoroacetyl-L-alanine*

For the preparative resolution of the $\pm$3,3-dimethyl-2-butyl-N-trifluoroacetyl-L-alanine diastereomers, the 1584-cm column was utilized. The column temperature was 165° and the nitrogen carrier flow rate was 230 ml/min. No flash heater was used. A 15-$\mu$l injection was introduced into the column every 6–7 min. Since the injected material had a retention time of approximately 30 min, approximately five injections were on the column at any given time after the fifth injection. Injection timing was varied slightly to facilitate concurrent injection and trapping schedules. The outlet tube to the cold trap was maintained at about 190°. The splitter was adjusted to deliver >99% of the chromatographed material to the cold trap allowing the remainder to go to the hydrogen flame detector. The trapping schedule could thus be synchronized to the actual elution by watching the chart recorder. Trapping for the first peak was initiated after the recorder pen began to rise, and was terminated slightly before it reached the low point between the two peaks. Trapping for the second peak was initiated shortly after the pen began to rise and terminated as the pen reached base line (Fig. 3).

*Saponification of the resolved diastereomers*

Small portions (0.1–0.3 ml) of the resolved diastereomers were placed in separate 100 × 13 mm screw cap culture tubes. A 20% (w/v) methanolic NaOH solution made of methanol–water (7:5) was added to each diastereomer until the final NaOH molarity was three times that of the diastereomer. Boiling chips were added and the tubes sealed with Teflon-lined caps and immersed to the level of the contents in a boiling water bath for 1 min. The saponification mixture was cooled under tap water, diluted to twice its volume with water and extracted quantitatively with equal volumes of ether. The pooled ether extracts were then washed with water until neutral and dried over anhydrous $Na_2SO_4$. The resolved 3,3-dimethyl-2-butanol was conveniently separated from the ether and methanol by preparative gas chromatography using a 1 × 528 cm column packed with 10% OV-17 on 45/60 mesh DMCS-treated acid-washed Chromosorb W.

For larger amounts the saponification time may need to be increased. Trial saponifications may be conveniently monitored on unactivated Silica Gel G thin-layer plates, chromatographed in the top phase of *n*-butanol–acetic acid–water (25:6:25) and developed with ninhydrin. The trifluoroacetyl moiety is removed almost immediately and the resulting ester is readily detected with ninhydrin as is alanine, one of the final hydrolysis products. The $R_F$ value of the alanyl ester is about 4.7 times that of alanine. The disappearance of the alanyl ester may also be monitored by gas chromatography on 3-m 10% OV-1 analytical columns.

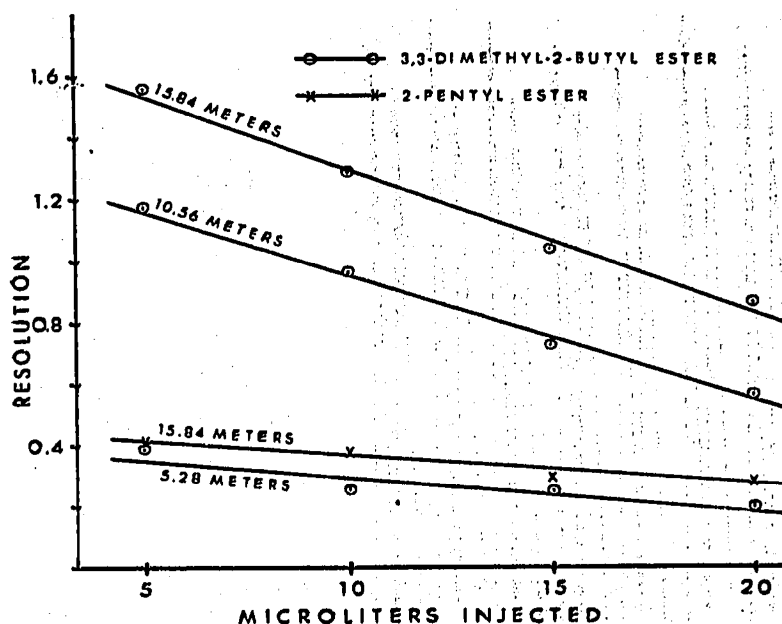


Fig. 2. The effect of length of the preparative column on separation of the two diastereomers of 3,3-dimethyl-2-butyl-N-trifluoroacetyl-L-alanine (⊙—⊙) and 2-pentyl-N-trifluoroacetyl-L-alanine (×—×).

*Determination of optical purity*

To test the stearic purity of the resolved alcohols the (+) or (—) 3,3-dimethyl-2-butyl-N-trifluoroacetyl-D-alanyl derivative was prepared by the following micro-procedure. D-Alanine (50 mg) was dissolved in 1 ml of trifluoroacetic acid. To this 1 ml of trifluoroacetic anhydride was added. After 15 min at room temperature the solvent was removed under a stream of nitrogen and the N-trifluoroacetyl-D-alanine was dissolved in 1 ml of methylene chloride. To this 1 ml of thionyl chloride was added and allowed to stand for 15 min at room temperature. The solvents were again removed under a stream of nitrogen and the acid chloride dissolved in 1 ml of methylene chloride. From this two 0.1-ml aliquots were taken and reduced to 0.02 ml under a stream of nitrogen in two small conical centrifuge tubes. Separately, 5 $\mu$l of the appropriate optical form of the alcohol was added to each tube. After several minutes 0.5 ml of methylene chloride and 0.5 ml of water were added to each of the samples and the aqueous fraction was extracted quantitatively with methylene chloride. The methylene chloride fractions from each sample were dried with a small amount of anhydrous $Na_2SO_4$, and evaporated to 0.1 ml under a stream of nitrogen. The derivatives were chromatographed on 3-m 10% OV-17/DMCS-treated acid-washed Chromosorb W columns at 118° with a nitrogen carrier gas flow rate of 90 ml/min. No flash heater was used.

## RESULTS

The effect of column length on the degree of separation is quite pronounced. This is shown in Fig. 2 by a plot of resolution *versus* the quantity injected. The number of theoretical plates, based on the first peak for 15-$\mu$l injections of unresolved material at 165° and a carrier gas flow rate of 230 ml/min, increased with column length,

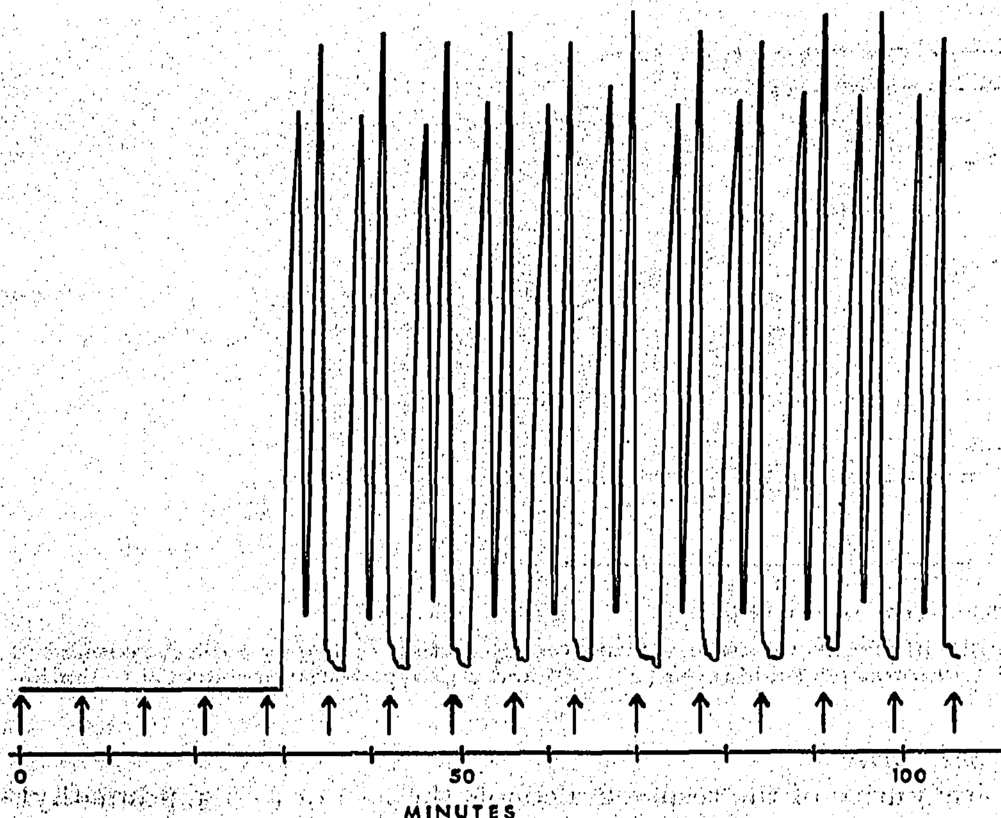


Fig. 3. Preparative separation of $\pm$ 3,3-dimethyl-2-butyl-N-trifluoroacetyl-L-alanine. Arrows indicate 15-$\mu$l injections. The first peak of each group of two represents the ester containing the levorotatory form of the alcohol and the second peak the dextrorotatory form. Chromatography was done on a 1 $\times$ 1584-cm column packed with 10% OV-1 on 45/60 mesh DMCS-treated acid-washed Chromosorb W at 165° and a nitrogen flow rate of 230 ml/min.

*i.e.* 553, 1345, and 2277. Fig. 3 shows the separations attained with the 1584-cm column at 165° for 15-$\mu$l injections, spaced approximately 6 min apart. Slightly better separations can be obtained at lower temperatures with all three column lengths, but since the peak bases are spread out more, the time interval between injections must be increased and the ratio of the amount injected/unit of time is diminished. As can be seen from Fig. 2, the ability of the 1584-cm column to resolve the 2-pentyl diastereomer is much less than its ability to resolve the 3,3-dimethyl-2-butyl analogue. Slightly better separation was obtained with the 2-octyl analog. Because of difficulties encountered in preparing large quantities of the 1,1,1-trifluoro-2-propyl derivative, only slightly larger than analytical amounts (*ca.* 10 $\mu$g) were tested. The degree of separation was approximately the same as that given by a similar load of the 3,3-dimethyl-2-butyl analog.

Fig. 4 shows the chromatograms of the esters formed by esterification of N-trifluoroacetyl-D-alanine with one of the resolved 3,3-dimethyl-2-butanols and thus indicates the degree of resolution achieved by the preparative column. It should be pointed out, however, that the results depicted in Fig. 4 indicate the minimum degree of resolution achieved because of the possibility of small amounts of racimization of the alcohol during the saponification as well as of the alanine during the formation

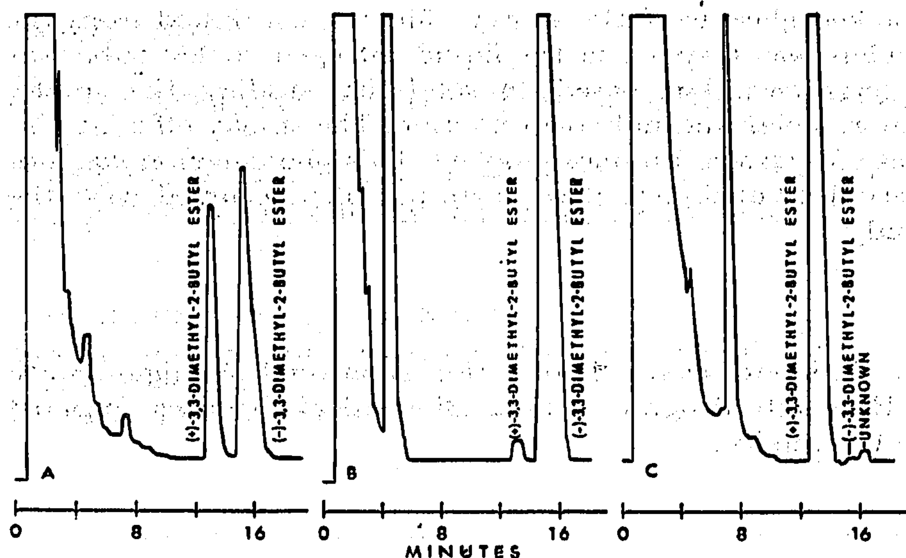


Fig. 4. Gas chromatographic demonstration of the optical purity of the 3,3-dimethyl-2-butanol attained by preparative gas chromatography. The N-trifluoroacetyl-D-alanyl esters were chromatographed on a 3-m 10% OV-17 on 100/120 mesh DMCS-treated acid-washed Chromosorb W column at 118° and a nitrogen flow rate of 90 ml/min. Chromatogram A is from the derivatives made from unresolved alcohol; chromatograms B and C are from derivatives made from alcohols derived respectively from the first and second peaks of the preparative chromatography.

of the N-trifluoroacetyl acid chloride. It is also possible that the D-alanine used for the esterification was contaminated with small amounts of the L-isomer. By triangulating the peaks of the more prevalent diastereomers and comparing the peak areas of the two diastereomers, the optical purities are shown to be $>98\%$.

Polarimetric studies showed the alcohol derived from the first peak from the preparative column to be levorotatory and from the second to be dextrorotatory. The last peak in Fig. 4C is apparently not the result of contamination by the levorotatory form and is of unknown origin, but perhaps represents a structural isomer or analog of 3,3-dimethyl-2-butanol. The contamination due to the levorotatory form is apparently represented by the shoulder preceding the unknown peak. The slight tailing in Fig. 4A probably is also the result of this unknown material.

## DISCUSSION AND CONCLUSIONS

With the aid of automatic injection and collecting devices and improved columns, especially longer columns, preparative gas chromatographic resolution of at least certain alcohols would be quite practical. The maximum practical column length for this purpose is not known, but the results indicate that it was not surpassed in this study.

The use of an improved cold trap would also be beneficial. By using the trap described, only about 62% of the amount injected could be accounted for in the trap. Although nearly all the trapped material was condensed on the first 2 or 3 cm of the trap, quantities of smoky-appearing material issued from the cold trap outlet. The appearance of this material coincided with the elution of the two diastereomers. Some of this material could be trapped in a U-tube cooled by liquid nitrogen but even with

several inches of loosely packed glass wool the smoky effluence continued from the trap outlet. A portion of what was trapped in the liquid nitrogen cooled tube was the 3,3-dimethyl-2-butyl derivative, but based on solubility studies, the smoky material also contained a more polar and unknown fraction. The smoky effluent also appeared when trapped material was rechromatographed. This phenomenon was not an artifact of the 3,3-dimethyl-2-butyl derivative alone but also occurred with the other esters tested in this study.

ACKNOWLEDGEMENTS

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REFERENCES

1 A. W. INGERSOLL, *Organic Reactions*, Vol. II, John Wiley, New York, 1944, pp. 376–414.
2 G. E. POLLOCK AND V. I. OYAMA, *J. Gas Chromatog.*, 4 (1966) 126.