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## Note

# Simple gas–liquid chromatographic technique for the analysis of muramic acid

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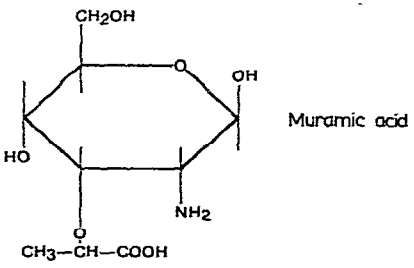
The peptidoglycan moiety of bacterial cell walls consists of amino acids, amino sugars and muramic acid[1]. There are numerous biochemical interests in the peptidoglycan moiety as it relates to the rigidity and shape of the bacterial cell wall. Muramic acid has a special interest to some investigators as it is a compound which is unique to bacteria. Our investigations of peat-forming systems and the role of microorganisms as contributors of organic constituents to peat has focused our attention on the merits of studying muramic acid levels in peat.




Muramic acid

Methods have been published for the analysis of muramic acid via paper chromatography[2], ion-exchange[3] (amino acid analyzer) and thin-layer chromatography[4]. Moss *et al.*[5] used the N-heptafluorobutyl *n*-propyl ester derivative and subsequent gas chromatography for the analysis of muramic acid. Our experience with this procedure and that for the *n*-butyltrifluoroacetyl *n*-butyl esters of amino acids has shown the derivatization procedure to be somewhat tedious[6].

Muramic acid is essentially a carbohydrate moeity containing a number of hydroxyl functions; it was felt that a less time-consuming derivatization method such as silylation and subsequent gas–liquid chromatographic (GLC) analysis could be used in place of the N-heptafluorobutyl *n*-propyl ester derivatives.

## EXPERIMENTAL

A stock solution of muramic acid (Sigma, St. Louis, Mo., U.S.A.) was made

using silylation grade pyridine (Pierce, Rockford, Ill., U.S.A.). Aliquots of the stock solution were pipetted into 5-ml septum bottles; after placing the bottle in a water-bath at 35°, the solution was evaporated to dryness using nitrogen. One milliliter of Tri-Sil Z (Pierce) which is a solution of trimethylsilylimidazole in pyridine (1:1), was added to the septum bottle. After sealing the bottle with a Tuf-Bond (Pierce) PTFE–silicone septum, the sample was shaken for 30 sec to dissolve the muramic acid; the contents were allowed to react for a minimum of 10 min before GLC analysis.

The derivatized muramic acid was chromatographed using a flame-ionization detector on a Tracor 222 gas–liquid chromatograph; a 2 × 4 mm I.D. glass column packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.) was used for analysis. A programming rate of 4°/min between 175° and 225° with an initial programming delay of 2 min plus a flow-rate of 40 ml/min (nitrogen) was found to be adequate. Injection port and detector temperatures were 250 and 275°, respectively. Peak integration was accomplished by using a Hewlett-Packard 3380A integrator.

## RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram that represents a typical GLC analysis; the two peaks for muramic acid are due to the $\alpha$ and $\beta$ anomeric forms. The small peaks are

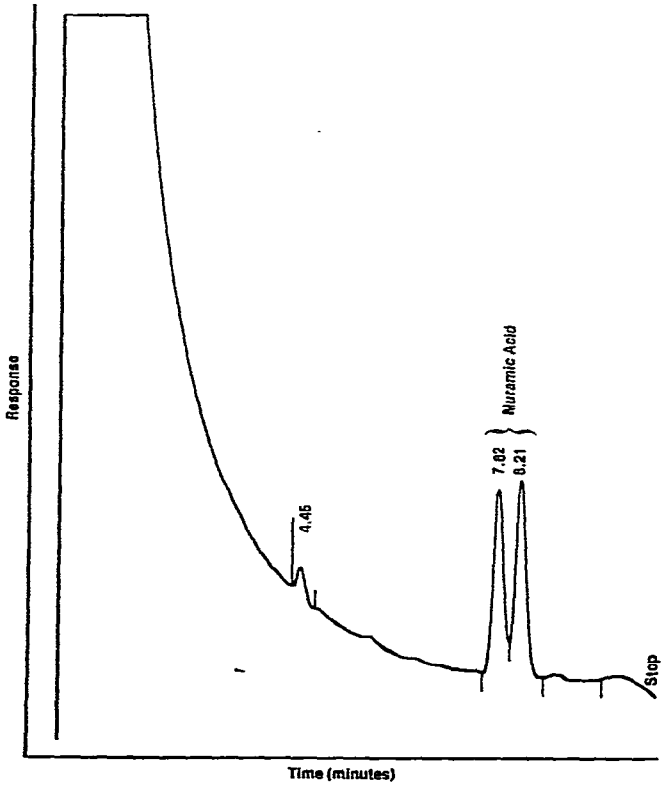



Fig. 1. Chromatogram showing muramic acid analysis. For conditions, see under Experimental.

primarily due to derivatizing reagents and perhaps some breakdown products. Fig. 2 shows a standard curve for muramic acid that represents approximately a three-order-of-magnitude dynamic range; the standard deviation was 1.4 to 6.8% from the high to low concentration end of the range.

In addition to the speed of the analysis, a further advantage in using a trimethylsilylimidazole–pyridine (1:1) solution over other varieties is that according to the manufacturer's (Pierce) specifications the sample solution could be as much as 50% aqueous without affecting the potency of the derivatizing reagent.

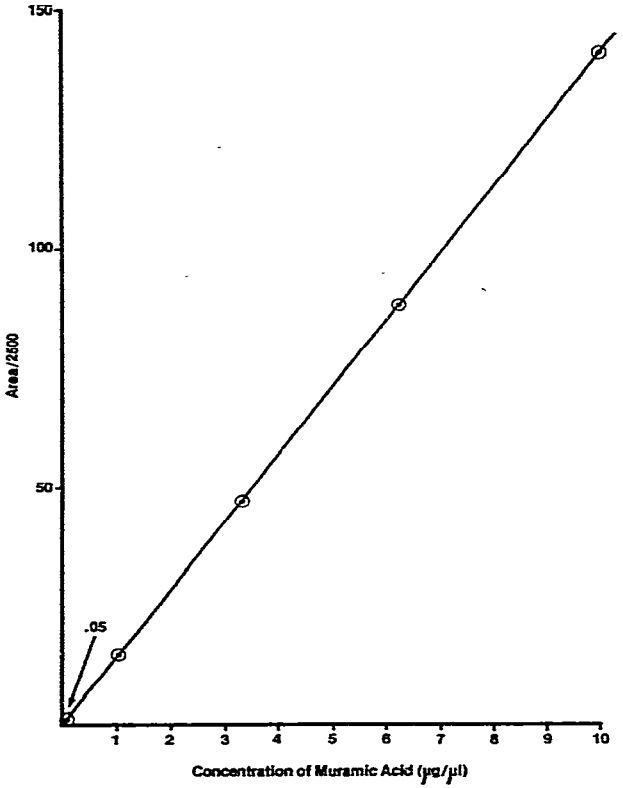



Fig. 2. Standard curve for muramic acid.

## CONCLUSION

A method has been developed for the analysis of muramic acid, which is considerably faster and simpler than methods previously published. The method appears to have adequate sensitivity and reproducibility for a wide range of interests.

## ACKNOWLEDGEMENTS

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