1. Blomstrand, R., Gürtler, J. and Werner, B. *Acta Chem. Scand.* **18** (1964) 1019.
2. Blomstrand, R. and Dahlbäck, O. *Acta Soc. Med. Upsalien.* **54** (1959) 177.
3. James, A. T. and Piper, E. A. *J. Chromatog.* **5** (1961) 265.
4. Cacace, F. *Nucleonics* **12** (1961) 45.
5. Blomstrand, R. and Gürtler, J. *Acta Chem. Scand.* **18** (1964) 276.

Gas Chromatography of β-Hydroxybutyric Acid and Biochemically Related Compounds

LARS HAGENFELDT and ROLF BLOMSTRAND

Department of Clinical Chemistry at Serafimerlasarettet, Karolinska Institutet, Stockholm, Sweden

In metabolic studies of diabetes mellitus there is a great need for specific methods for the quantitative determination of the ketone bodies (acetone, β-hydroxybutyric acid, and acetoacetic acid) and related metabolic intermediates [1] (*e.g.* pyruvic and lactic acids and the components of the tricarboxylic acid cycle). The available biochemical methods for the determination of the ketone bodies are cumbersome and unsatisfactory recoveries are obtained, especially with regard to β-hydroxybutyric acid.[2]

No satisfactory gas chromatographic technique has yet been published for separation of the ketone bodies and related compounds. During the last years there has, however, appeared a few reports on the separation of the biologically important di- and tricarboxylic acids.[3-5] In the present report a study has been made of the gas chromatographic separation of β-hydroxybutyric, acetoacetic and related acids, and the quantitative aspects of this analysis has been investigated.

Experimental. An Aerograph Hi−Fi 600 Gas Chromatograph with a flame ionization detector was used together with a 1 mV potentiometric recorder (Servo/riter, Texas Instruments Inc.). Coiled glass columns, 1500 mm long and with 2 mm I.D., were packed with 25 % by weight of Castorwax (Wilkens Instrument Inc.) on 100 to 120 mesh acid-washed and dimethyldichlorosilane-treated Chromosorb W. The columns were preconditioned overnight at 180°C. The following gas chromatographic conditions were employed: injection port temperature 250°C, oven temperature 125°C, flow rate of carrier gas (nitrogen) 25 ml/min and flow rate of hydrogen 25 ml/min.

The acids to be analyzed were dissolved in ether and converted to their methyl esters through the addition of an ethereal solution of diazomethane. A Hamilton microsyringe was used for the injection of the samples. The reference compounds used were of the highest purity commercially available and were in most cases gas chromatographically pure within reasonable limits.

Results. In Table 1 are listed the retention times of the methyl esters of some organic acids relative to the ester of β-hydroxybutyric acid. A good separation between the different acids can be achieved as illustrated in Fig. 1, which shows a chromatogram of four biologically important acids.

Table 1. Relative retention times of the methyl esters of some organic acids.
(β-Hydroxybutyric acid methyl ester = 1.00, 5.3 min)

Acetic 0.07; propionic 0.11; butyric 0.19; valeric 0.37; caproic 0.69; enanthic 1.30; octanoic 2.45.	
Lactic 0.42; β-hydroxybutyric 1.00.	
Pyruvic 0.30; α-ketobutyric 0.53; acetoacetic 0.76; α-ketocaproic 1.69.	
Oxalic 0.57; malonic 0.95; succinic 1.97.	

These four acids, pyruvic, lactic, acetoacetic, and β-hydroxybutyric acids, were also chosen for a quantitative study of the method. There is a linear relation between the amount of ester injected and the peak area recorded, which is illustrated for β-hydroxybutyric acid in Fig. 2. In Table 2 the results of three gas chromatographic analyses of a mixture of the four acids are presented compared to the weight composition of the mixture. In the quantitative experiments the peak areas were either compared to that of octanoic acid methyl ester as an internal standard or factors for the individual acids determined from a known standard mixture. Both methods gave satisfactory results.

Comments. These preliminary results indicate that it is possible to separate and quantitate a variety of low molecular

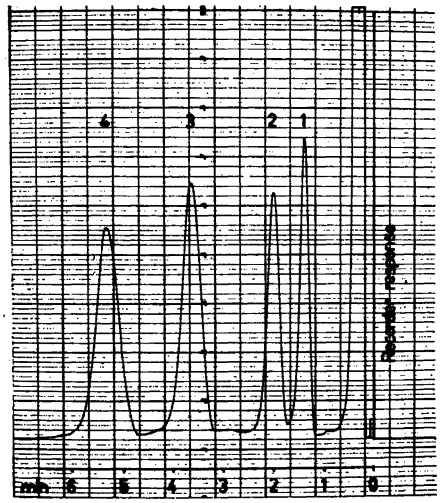

Fig. 1. Chromatogram of some organic acids related to β-hydroxybutyric acid. 1. pyruvic; 2. lactic; 3. acetoacetic and 4. β-hydroxybutyric acid. Photograph of original recorder tracing. *Conditions:* Coiled glass columns, 1500 mm long, 2 mm I.D. Chromosorb W, 100–120 mesh, acid-washed, silanized. 25 % by weight Castorwax. Temperature: injection port 250°C, oven 125°C. Carrier gas: nitrogen 25 ml/min. A flame ionization detector was used.

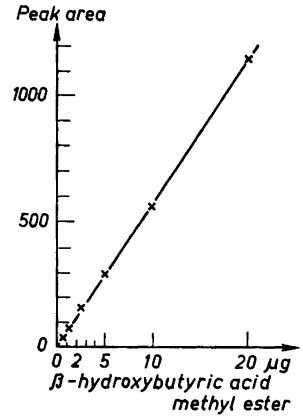

Fig. 2. Gas chromatographic analysis of β-hydroxybutyric acid shows a linear response of the flame ionization detector.

weight organic acids of biological importance. Through the application of temperature programming acids of higher molecular weight can be included in the analysis. The acids of the tricarboxylic acid cycle are well separated on the same column with the exception that malic and oxaloacetic acid overlap. An example of a temperature programmed analysis of

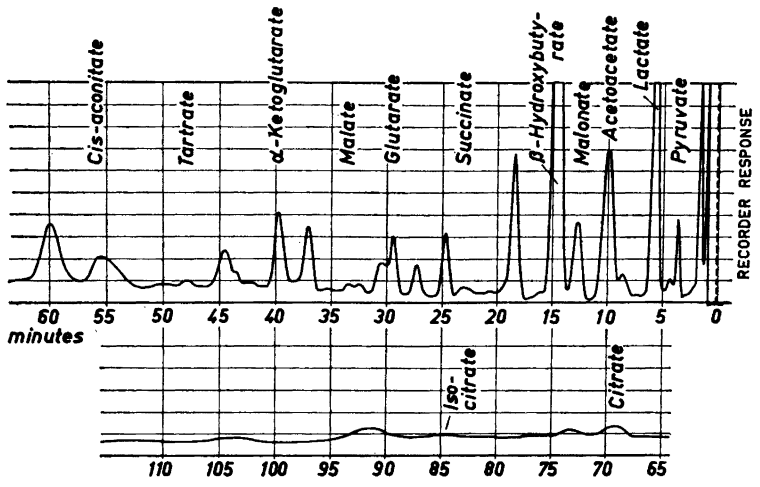

Fig. 3. Analysis of organic acids from the urine of a diabetic patient with a slight ketoacidosis. Oven temperature was programmed from 100° to 175°C at a rate of 2°/min. Otherwise the conditions were the same as in Fig. 1. The acids were purified by passing the urine through a Dowex 2-X8 column, eluted with 5 N NaCl and extracted with ethyl ether.

Table 2. Results of three gas chromatographic analyses (GLC) of a mixture of four organic acids compared with gravimetric determination.

Acid	Gravimetric determination Weight per cent (as methyl esters)	GLC determination I	II	III	Mean
Pyruvic	23.9	23.7	24.0	24.9	24.2
Lactic	28.9	28.3	29.6	28.5	28.8
Acetoacetic	19.8	20.9	19.7	19.6	20.1
β-Hydroxybutyric	27.4	27.1	26.7	27.0	26.9

organic acids from the urine of a diabetic patient with a slight ketoacidosis is shown in Fig. 3. In this chromatogram, however, several peaks still remain to be identified. The same technique has also been successfully applied for the determination of citrate in plasma from patients under various clinical conditions.

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1. Krebs, H. A. *Arch. Internal. Med.* **107** (1961) 51.
2. Johnson, R. E., Passmore, R. and Sargent, F. *Arch. Internal. Med.* **107** (1961) 43.
3. Kowala, C., Kranz, Z. H. and Murray, K. E. *Australian J. Chem.* **54** (1962) 832.
4. Luke, H. H., Freeman, T. E. and Kier, L. B. *Anal. Chem.* **35** (1963) 1916.
5. Kuksis, A. and Vishwakarma, P. *Canadian J. Biochem. Physiol.* **41** (1963) 2353.

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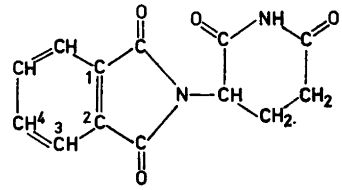
The Crystal Structure of N-(α-glutarimido)-4-bromophthalimide

SVEN FURBERG and
CHERRY SCHIANDER PETERSEN

Institute of Chemistry, University of Oslo, Blindern, Oslo 3, Norway

The glutarimido derivative of phthalimide (I) has various striking pharmacological activities. It would appear to be of importance to know its molecular structure, and we have therefore started X-ray crystallographic investigations of this and related compounds.

A sample of (I) was kindly supplied by Norsk Astra. It consisted of small well-developed crystals elongated along the *b* axis. The X-ray diagrams showed them to be monoclinic, with $a = 8.28$ Å,



(I)

$b = 10.07$ Å, $c = 15.37$ Å, and $\beta = 109°$ (all ± 1 %). By flotation the density was found to be about 1.42 g cm^{-3} and there are four (calc. 4.01) molecules in the unit cell. The space group is $P2_1/c$.

Although the space group is favourable, the crystallographic axes are all rather long and it might be difficult to determine the crystal structure. The 4-bromo-derivative of (I) was then prepared by Dr. Else Kloster-Jensen of this Institute, and we continued the investigation using this compound, in which the essential structural features of (I) must be expected to be retained. An account of the synthesis will be published separately.

Small single crystals were eventually obtained by sublimation in vacuum, and X-ray Weissenberg photographs taken around the *a* and *b* axes. The crystals were