한국농화학회지 Vol. 21, No. 1 March, 1978
J. Korean Agricultural Chemical Society

Gas chromatographic and mass spectrometric identification of alloisoleucine in maple syrup disease

Jo, Do-Hyun
Dept. Chem. Eng., Ajou Inst. of Technol. Suwon, Korea
(Received Nov. 2 1977)

Maple syrup 병환자 중 Alloisoleucine의 GLC 및 질량분석법에 의한 동정

조 도 현
아주공과대학
(1977년 11월 2일 수리)

SUMMARY

Alloisoleucine from the plasma of maple syrup disease affected patients was separated from isolecine and identified by gas liquid chromatography and mass spectrometry.

INTRODUCTION

The analysis of amino acid content by gas-chromatography became routine in the laboratories. In this latoratory the gas chromatographic determination of amino acid content was done withnorvaline as internal standard (IS) on two columns, one of which was filled with OV-17 and the other with XE-60 and OV-17(mixed phase). In the case of maple syrup disease affrected plasmas and urines the contents of all amino acids calculated on the mixed phase column were unusually lower than those obtained on the OV-17 column (1). The only explanation possible for this discrepancy was an interference of a compound having the same retention time as norvaline.

In this paper further investigations on this problem will be reported by using gas-liquid chromatography (GLC) and combined gas-liquid chromatography-mass spectrometry(GLC-MS)

EXPERIMENTS

(1) Derivatization into N-trifluoroacetyl (TFA), n-butyl esters

The derivatization was carried out according to the procedures developed by Gehrke's group (2) and modified by Barbier-Chapius (3) and Jo (4). The procedure can be briefly described as follows. The standard amino acid mixture and the sample were passed through a glass column (5 cm long and 0.5 cm in diameter) of AG 50 W-XI cation exchanger (Biorad. Lab., Richmond, Calif. USA) and eluted with a 4N-NH_4OH solu tion after washing with distilled water. They were dried under a nitrogen stream and esterified in a teflon-lined 10ml capped tube with a 2N HCl solution in n-butanol. After drying the esteri fied samples under a nitrogen atmosphere, they were acylated with a trifluoroacetic anhydride-dichloromethan mixture (1:9).

(2) GLC and GLC-MS analysis

GLC analysis were carried out with Packad 419 equipped with flame ionization detector. Glass columns of 3 m by 3 mm in diameter were filled as follows; The first one-third of the first column was filled with 2% XE-60 impregnated on Supelcoport (Supelco, Inc., Bellefonte, P.A. USA) and the remaining part with 1% OV-17 impregnated on Supelcoport. The second column was filled with CPM-G3107 (Coated Packing Material, Gallard Schleisigner Chem. Manuf. Corp, Carl Place, N.Y. USA). Inlet pressures of hydrogen, air and nitro gen were 1.00 atm, 1.25 atm and 2.00 atm respectively. The temperature of injection port was at 205°C and that of detector at 250°C The column temperature was initially held at 85°C for 5 min and programmed at 3°C/min to the desired temperature.

Combined GLC-MS analysis were done with a LKB-9000. Helium was used as the carrier gas and all of the mass spectra were obtained at 28 eV. Other operating parameters were; Injection port 210°C, molecular separator 280°C, ion source 200°C, accelerating voltage 3.5 KV and trap current 60μA. Mass spectrum recording was obtained by means of an oscillograph recorder.

RESULTS AND DISCUSSION

To the samples of maple syrup disease cycloleucine (cleu) was added as IS in stead of norvaline, which was otherwise used as IS. As seen in Fig. 1A and 1B, Peak 1 had the same retention time as norvaline on the mixed phase column. This verified the previous explanation why the content of amino acids calculated on the mixed column was lower than those on the OV-17 column.

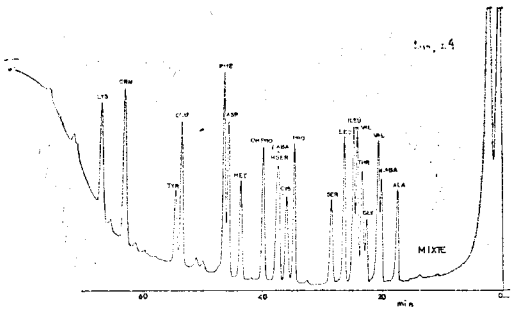

Fig. 1A: Chromatogram of the standard amino-acids mixture on the mixed phase column with norvaline as Is.

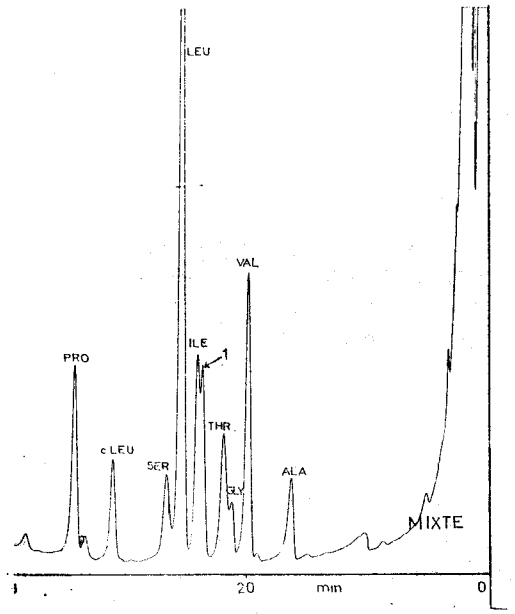

Fig. 1B: Chromatogram of the plasma of maple syrup disease affected patient. Cycloleucine as IS in stead of norvaline

Table 1: The comparison of fragments of isoleucine, leucine, norleucine and peak1

Aminoacids		Isoleucine M^+283	Leucine M^+283	Norleucine M^+283	Peak 1 in Fig. 2A
Fragments	m/e	RI %	RI %	RI %	RI %
M-56 (C_4H_8)	227	9.1	4.2	2.7	15.1
M-56 (C_4H_8) -56 (Side chain-H^+)	171	29.1	5.5	3.7	33.9
M-57 (Side Chain) -73 (C_4H_9O)	153	35.5	8.6	5.6	36.9
M-143	140	2.3	43.6	8.1	2.8
M-157	126	8.2	4.4	19.7	8.6

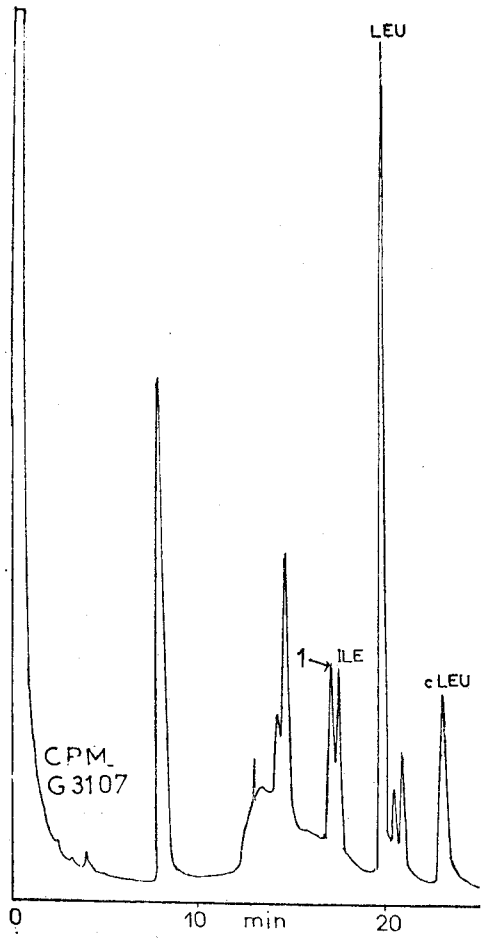

Fig. 2A: Chromatogram of the same sample analy-zed in Fig. 1B on the CPM-G3107 column.

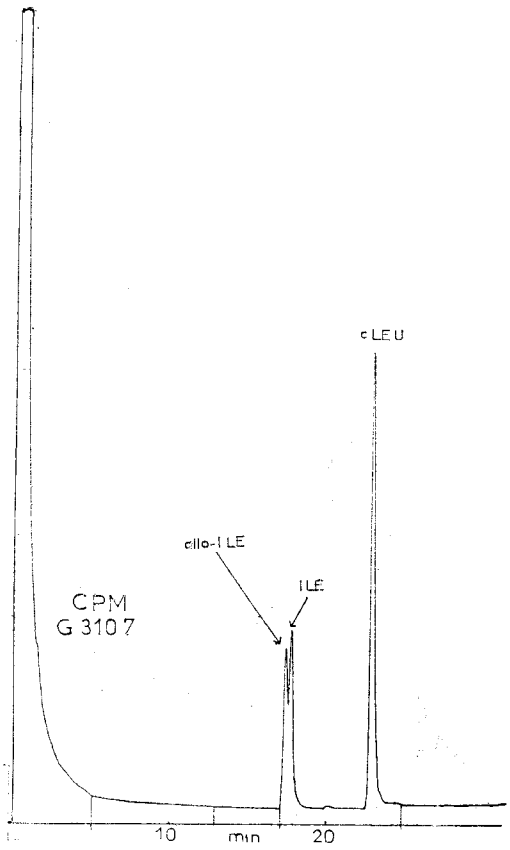

Fig. 2B: Chromatogram of the standard alloisoleucine on the same column.

For the better separation of Peak 1 from isoleucine, the same sample was chromatographied on the CPM-G3107 phase (Fig. 2A). On this condition a mass spectrum of this compound could be obtained and shown in Table 1 with isoleucine, norleucine, leucine. By comparing the relative intensity of fragments m/e 227, 171, 153, 140 and 126 the peak was assumed as an other isomer of isoleucine, alloisoleucine. For the standard of alloisoleucine only the mixture of alloisoleucine. and isoleucine(approximetely 1 : 1)(Sigma Chem., St Louis. Mo. USA) was available at the time The chromatogram of alloisoleucine was obtained from the mixture (Fig. 2B). The peak 1 and authentic alloisoleucine showed the same retention time on the CPM-G3107 phase, (Fig. 2A and Fig. 2B)

Fig. 3A and Fig. 3B showed the mass spectrum of peak 1 and the standard alloisoleucine. The fragment of m/e=182, M−101 ($-\overset{\overset{O}{\|}}{C}-O-C_4H_9$) was the highest abundant ion in the both compounds. By comparing the other fragments, m/e=227, M−56(C_4H_8), m/e=171, M−56−56 (C_4H_8, $\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH\text{-}H^+$) m/e=153, M−57−73 ($\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH$, C_4H_9O), the relative intensity of those fragments were almost same in Peak 1 and the authentic alloisoleucine standard. The peak 1 was definitively identified as alloisoleucine.

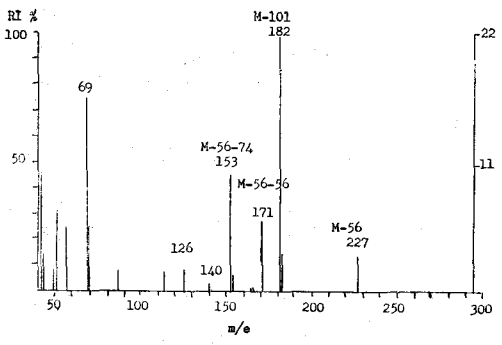

Fig. 3A: The mass spectrum of peak 1.

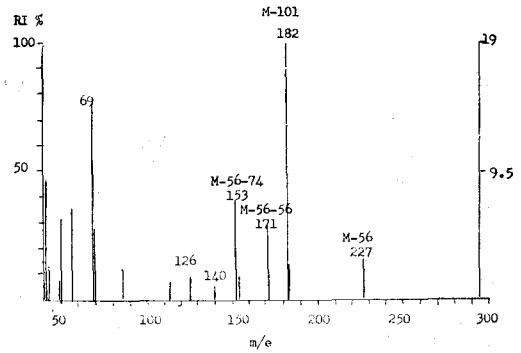

Fig. 3B: The mass spectrum of the authentic alloisoleucine.

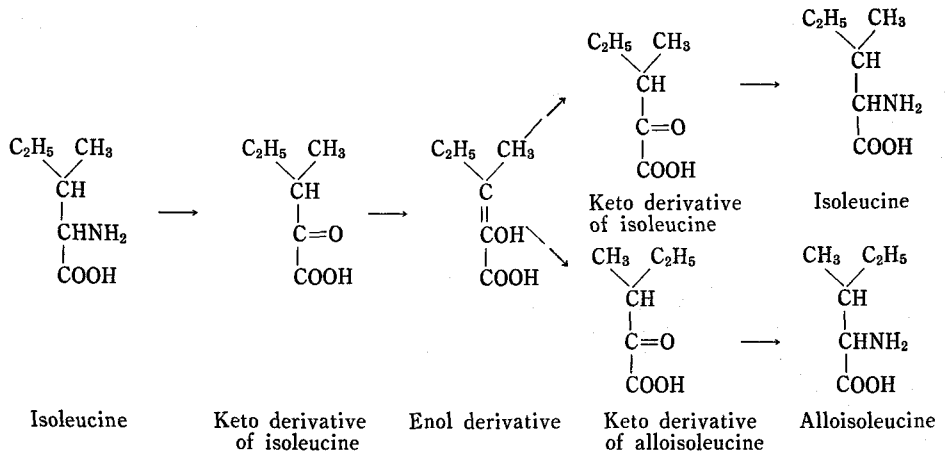

Fig.4:The pathway for the formation of alloisoleucine from isoleucine

The formation of alloisoleucine, diastereoisomer of isoleucine, can be attributed to enolization of the keto acid derivative of isoleucine and subsequent reamination (Fig.4). This is secondary to the high level of keto acid present as a result of the failure of decarboxylation after transamination of the ramified aminoacids. This failure is characteristic to the maple syrup disease.

With the aid of ion exchange chromatography the occurence of alloisoleucine was already reported by Piez and Morris(5) in 1960 and Norton et al (6). in 1962 By gas chromatography Darbre and Blau (7) in 1965 partially separated alloisoleucine from isoleucine in the standard mixture of amino acids under the form of N-TFA, n-amyl esters. In 1973 Cliffe et al(8) obtained a better separation of the two isomers as N-TFA, methyl ester derivatives on a mixed phase (XE-60, OF-1 and MS-200). But they also used the mixture of standard amino acids. Williams and Halperns (9) in 1974 analys ed the plasmas of maple syrup disease and in the same year Adams(10) did analyze urines of the same disease affected patients by GLC with or without using MS. But they did not report the presence of alloisoleucine which is characteristic for the disease.

It appeared very important for the correct treatment of the disease to be able to determine the quantity of this amino acid in plasma as well as in urine by gas chromatography.On the other hand the D or L configuration of this amino acid remained to be determined but may be resol ved in the near future by the resolution techni ques reported by Bonner et al (11).

ACKNOWLEDGEMENT

The author wishes to express his deepest appre ciations to Prof. Padieu, P. and Dr. Desgres, J.for guidance and inspriation.

요 약

측쇠 아미노산의 대사의 장해를 받음으로 생기는 maple syrup disease환자의 혈액에서 alloisoleucine를 GLC와 질량분석기를 사용하여 동정하였다.

REFERENCES

1. JO, Do-Hyun and Desgres J., Unpublished data
2. Kaiser, F.E., Gehrke, C.W., Zumwalt, R.W. and Kuo, K.C., J. Chromatogr., **94**, 113 (1974)
3. Barbier-Chapius, F., Lavoue, G. and Padieu, P., Anal. Biochem., Under press (1977)
4. JO, Do-Hyun, Diplome D'etudes Approfondies (1975), Fac de Sci., Univ. de Dijon, France
5. Piez, K.S. and Morris, L., Anal. Biochem., **1**, 187 (1960)
6. Norton, P.M., Roitman, E., Snyderman, S.E. and Holt, J.E. Jr., Lancet, **1**, 26 (1962)
7. Darbre, A. and Blau, K., J. Chromatogr., **17**, 31 (1965a)
8. Cliff, A.J., Berridge, N.J. and Westgarthe, D.R., J. Chromatogr., **78**, 333 (1973)
9. Williams, K.W. and Halpern, B., Aust. J. Biol. Sci., **26**, 831 (1974)
10. Adams, R.E., J. Chromatogr., **95**, 189(1974)
11. Bonner, W.A., Van Dort, M.A. and Flores, J.J., Anal. Chem., **46**, 2104 (1974)