A Handy Method of Determining Relative Amounts of DL-Threonine and DL-Allothreonine with the Aid of Infrared Absorption Spectroscopy

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In the preparation of threonine, mixtures of the diastereoisomers, DL-threonine (I) and DL-allothreonine (II), are produced and the selective preparation of one isomer is considered to be difficult. Thus, it is very important to be able to determine the relative amounts of I and II by a simple method.

The authors have found a simplified method to determine the relative amounts of I and II with the aid of infrared absorption spectra in the potassium bromide region. Many quantitative analytical methods for I and II have previously been reported, e. g., paper chromatography[1], ion exchage resin chromatography[2], 2, 4-dinitrophenyl derivative chromatography[3], and microbial assay[4]. Of these, the last one is probably the most reliable[5], but the procedure is still limited in scope.

Concerning the infrared absorption spectra of threonine, there have been some communications[6-8], but they show only the rock salt region.

In the present communication, the infrared absorption spectra of the potassium bromide region are dealt with; also, a simplified calculation method with absorption spectra at 702 cm^{-1} and 619 cm^{-1} by the potassium bromide tablet method is described[9]. The method was found experimentally and was not so much affected by the sizes of the sample granules[10]. Measurements were carried out as usual for qualitative analysis as absorptions appeared in the range between 20 and 80 per cent in transmission[11]. The calculation was

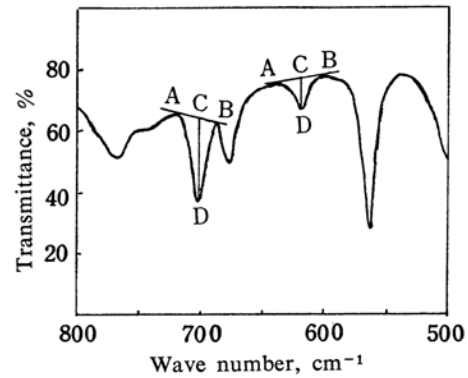

Fig. 1. IR absorption spectra of authentic mixture, No. 5.

1) K. N. F. Shaw and S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).
2) A. T. Shulgin, O. G. Lien, Jr., E. M. Gal and D. M. Greenberg, ibid., **74**, 2427 (1952).
3) T. Seki, *J. Biochem.*, 47, 235 (1960).
4) J. L. Stokes, M. Gunness, I. M. Dwyer and M. C. Caswell, *J. Biol. Chem.*, **160**, 35 (1945); M. Gunness, I. M. Dwyer and J. L. Stokes, ibid., **163**, 159 (1946).
5) K. Pfister, 3rd, E. E. Howe, C. A. Robinson, A. C. Shabica, E. W. Pietrusza and M. Tishler, *J. Am. Chem. Soc.*, **71**, 1096 (1949).
6) R. J. Koegel, J. P. Greenstein, M. Winitz, S. M. Birnbaum and R. A. McCallum, ibid., **77**, 5708 (1955).
7) K. Kodera, Y. Sato and T. Takahashi, *Yakugaku-Kenkyu*, **30**, 38 (1958).
8) K. Kodera, the 7th Infrared and Raman Spectra Conference, Osaka (1960), p. 173; T. Yamazaki and T. Takenishi, ibid. (1960), p. 176. In the above communications, quantitative analytical methods of allo/threo are given with absorption spectra at 14.26μ and 14.83μ, and at 870 cm^{-1} and 835 cm^{-1} respectively. However, the calculation is rather complicated.
9) A double-beam Hitachi infrared spectrophotometer Type EPI-2 was used.
10) S. Tanaka and M. Ogawa, *Japan Analyst*, **6**, 285 (1957). In this paper, Tanaka and Ogawa reported that the size of the sample granules had an effect on the optical density.
11) D. Z. Robinson, *Anal. Chem.*, **23**, 273 (1951).

as follows: In Fig. 1, the base line AB is drawn, and then CD lines are drawn vertically to the zero line at 702 cm^{-1} and 619 cm^{-1}. The CD lines at 702 cm^{-1} and at 619 cm^{-1} are measured with a rule ($\overline{CD}$(702 cm^{-1}) and $\overline{CD}$ (619 cm^{-1}) respectively) and the ratio is calculated.

Thus, the percentages of threonine and allothreonine are obtained:

$$\text{Percentage of threonine} = \frac{\overline{CD}(702\ cm^{-1})}{\overline{CD}(702\ cm^{-1}) + \overline{CD}(619\ cm^{-1})}$$

$$\text{Percentage of allothreonine} = \frac{\overline{CD}(619\ cm^{-1})}{\overline{CD}(702\ cm^{-1}) + \overline{CD}(619\ cm^{-1})}$$

The results obtained for authetic mixtures are presented in Table I. Authentic mixtures of threonine and allothreonine were prepared, using pure DL-threonine and DL-allothreonine. The purity of each substance was examined with infrared absorption spectra in the rock salt region[6], elementary analysis[12], paper chromatography[1] and melting point. From Table I, more favorable results were obtained when the content of DL-allothreonine in relation to DL-threonine was smaller. This method was applied to the synthesized sample. The synthesis was carried out according to the procedure reported by Sato et al.[13] The molar percentages of the threonine and allothreonine synthesized[14] were determined by this method; values of 77.6% of threo and of 22.4% of allo were obtained. The microbial assay method gave values of 78.9% of threo and of 21.1% of allo[15].

TABLE I. RESULTS OBTAINED FOR AUTHENTIC MIXTURES

NO.	A		B	
	threo-	allo-	threo-	allo-
1	95.0	5.0	95.2	4.8
2	93.7	6.3	93.8	6.2
3	84.3	15.7	84.7	15.3
4	83.3	16.7	82.3	17.7
5	74.6	25.4	74.2	25.8
6	53.9	46.1	54.9	45.1
7	48.6	51.4	47.3	52.7
8	35.4	64.6	37.9	62.1
9	11.5	88.5	12.3	87.7

A: Percentages of DL-threonine and DL-allothreonine in authentic mixtures.
B: Percentages determined by infrared absorption spectroscopy.

The bands which appear at 702 cm^{-1} and 619 cm^{-1} also abide by Beer's law in authentic mixtures, so the present authors are also investigating quantitative analysis on the basis of this fact. Details will be presented hereafter.

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12) Found for DL-threonine: C, 40.45; H, 7.84; N, 11.40, for DL-allothreonine: C, 40.48; H, 7.49; N, 11.60. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76%.

13) M. Sato, K. Okawa and S. Akabori, This Bulletin, 30, 937 (1957).

14) Found: C, 40.86; H, 7.65; N, 11.63. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76%.

15) We are indebted to Dr. J. Abe and Miss. Y. Shimojima for their microbial assays with *Streptococcus feacalis* R (ATCC 8043).