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Gas Liquid Chromatography of Alkaloids. II.[1] Quantitative Analysis of Alkaloids of *Lycoris radiata* Herb.

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A method for the quantitative determination of lycoramine and galanthamine in the bulbs of *Lycoris radiata* Herb. by gas liquid chromatography using a HI-EFF 8B column was described.

And the change of the amounts of these alkaliods were analyzed in every months through a year.

Most of *Lycoris* alkaloids which have been isolated as pure compounds were well known and their structures have been established. But the isolation and quantification of individual *Lycoris* alkaloid are tedious and time-consuming. In 1948, the quantitative estimation of Lycorine was achieved by isolation of the alkaloid as crystals.[3] And in 1960, galanthamine was determined quantitatively by ultraviolet (UV) absorption spectra.[4] In a previous paper,[1] a method for determination of alkaloids in *Lycoris radiata* Herb. was described.

The present report describes a procedure by which some individual components of *Lycoris* alkaloids can be accurately quantified by gas liquid chromatography.

Experimental

Plant exrtact——The bulbs of *Lycoris radiata* Herb. were collected at Yamato-Takada city (Nara) in every month, and cut and dried to constant weight. The dried material was pulverized and extracted with 99% ethanol (3 liters for 2 kg of dried matter, reflux for 15 hr, repeat 3 times), and the solvent was evaporated to dryness. The ethereal solution of the residue was extracted three times with 5% hydrochloric acid. The acidic aqueous solution was treated with solid sodium carbonate, and this basic solution was extracted again with chloroform. At this time the insoluble precipitate containing crude lycorine was collected.

The alkaloid extract was obtained by evaporating the solvent from the above chloroform solution.

Trimethylsilanized reagent was added to the anhydrous pyridine solution (5 ml) containing 100 mg of the alkaloid extract and 10 mg of chrysene, and then, an aliquot of this reaction mixture was chromatographed.

Gas Liquid Chromatography——All gas liquid chromatographical analyses were carried out an instrument equipped with a hydrogen flame ionization detector (Shimadzu model GC-1C). Columns were used with acid washed and silanized Gas Chrom P (60—80 mesh) coated with 3% film of HI-EFF 8B in 2.6 m glass U shaped tubes, 4 mm diameter, respectively. Column temperature was 240°. Nitrogen was used as carrier gas at flow rate of 66 ml/min. All quantitative works were performed by comparison of area responses with these of a known additions of chrysene as an internal standard.

Standard Curve——In each of several glass tubes were placed 10 mg of chrysene and known amounts of alkaloid ranging from

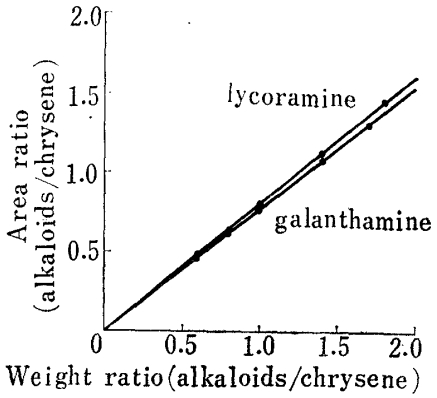

Fig. 1. Standard Curves for Lycoramine and Galanthamine with an Internal Standard (Chrysene)

1) S. Takagi, T. Katagi, and K. Takebayashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1116 (1968).
2) Location: *4-16, Edagawa-cho, Nishinomiya, Hyogo.*
3) M. Tomita, S. Ueo, T. Yonezawa, and M. Nakanishi, *Yakugaku Kenkyu*, **20**, 8 (1948).
4) K. Yamaguchi, H. Ogawa, and S. Natori, *Eisei Shikensho Hokoku*, **80**, 17 (1962).

5 to 20 mg. The materials were dissolved in 5 ml of anhydrous pyridine. To this solution were added 0.1 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. The tubes with glass stoppers were kept at room temperature. After 30 minutes, the reaction mixtures were applied on 3% HI-EFF 8B column. The peak areas were measured with a planimeter, each area reading being based on the average obtained from the cumulative value of five consecutive tracings. The weight ratio of alkaloid to chrysene was plotted against the corresponding ratio for the peak areas. A linear relationship was obtained as shown in Fig. 1.

Results and Discussion

Relative Molar Response

Relative molar response was determined for the TMS ethers of lycoramine and galanthamine using the polyester column, HI-EFF 8B, and the non-polar column, SE-30. These results are shown in Table I. The relative molar response for the TMS ethers of the alkaloids using the polyester column at 240° were essentially the same value using the non-polar column at 220°, showing no interaction between the alkaloids with polyester phase.

Table I. Relative Molar Response for Lycoramine and Galanthamine to Chrysene

	3% SE-30 220°	3% HI-EFF 8B 240°
lycoramine	1.00	1.04
galanthamine	0.97	0.97

Recovery

To an extract sample (*ca.* 100 mg) was added a known amount of alkaloid (*ca.* 10 mg). The samples were treated as described above. The results of these recovery tests were tabulated in Table II. Recoveries of lycoramine and galanthamine were 96, 99% respectively.

Table II. Recovery of Added Quantities of Lycoramine and Galanthamine to the Extract obtained from the Bulbs of *Lycoris radiata* Herb.[a]

Alkaloid extract (obtained in Feb.) (mg)	Lycoramine (mg)		Galanthamine (mg)	
	Added	Found	Added	Found
100.0	—	15.4	—	7.2
	10.0	25.0	10.0	17.1
recovery		96%		99%

a) Each value is the average of triplicate analyses.

Quantitative Change of Lycoramine and Galanthamine through a Year

The amounts of lycoramine and galanthamine in the bulbs of *Lycoris radiata* Herb. in every month were determined.

The results were shown in Table III and Fig. 2 in which each value was the average of five times repeated analyses.

As shown in Fig. 2, the amounts of lycoramine and galanthamine were changed similarly to each other through a year, and shown the minimum values in March and April, the months

Table III. Quantitative Determination of Lycoramine and Galanthamine in the Bulbs of *Lycoris radiata* Herb.

	Amount of Alkaloid (%)		Ratio of lycoramine per galanthamine
	Lycoramine	Galanthamine	
Oct.	0.0360	0.0196	1.84
Nov.	0.0490	0.0204	2.40
Dec.	0.0409	0.0175	2.34
Jan.	0.0295	0.0126	2.34
Feb.	0.0290	0.0134	2.16
Mar.	0.0176	0.0114	1.54
Apr.	0.0191	0.0136	1.40
May	0.0274	0.0188	1.45
Jun.	0.0394	0.0190	2.07
Jul.	0.0422	0.0194	2.17
Aug.	0.0356	0.0210	1.70
Sept.	0.0350	0.0208	1.68

of defoliation, and the maximum value in November, after flowering season. The change of the content of lycoramine in the bulbs were more remarkable than that of galanthamine, so the ratio of lycoramine per galanthamine were also changed through a season, and this maximum value was *ca.* 2.4 in November, and the minimum value was 1.4 in April.

As described above, in conclusion, the quantitative analysis of alkaloid in the bulbs of *Lycoris radiata* Herb. was achieved accurately and quickly. And this fact will contribute to a progress of a biogenetic and taxonomic studies.

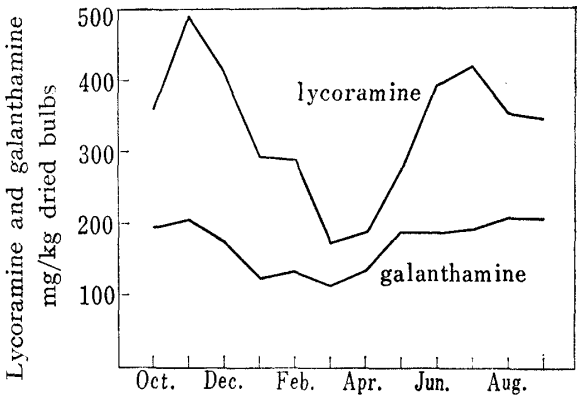

Fig. 2. The Annual Change of the Contents of Alkaloids in the Bulbs of *Lycoris radiata* Herb.