[Chem. Pharm. Bull. 16(6)1116—1120(1968)]

UDC 547.94.05 : 543.544.25 : 582.572.4

Gas Liquid Chromatography of Alkaloids. I. Separation of Alkaloids of Amaryllidaceae

Shuzo Takagi, Toyoshi Katagi, and Keiko Takebayashi

Faculty of Pharmaceutical Sciences, Mukogawa University[1]

(Received December 14, 1967)

A method for the separation of alkaloids of Amaryllidaceae by a gas chromatographic technique is described. The separation of trimethylsilyl derivatives of the alkaloids was carried out using a number of commercially available non-polar and polar stationary phases, with a hydrogen flame ionization detector system. An excellent resolution of a 12 components mixture was obtained with an HI-EFF 8B column. Application of this method to the extract of the alkaloids of *Lycoris radiata* Herb. was also investigated.

Gas liquid chromatography is now widely used for qualitative and quantitative analyses of alkaloid mixtures, and non-polar silicone polymers are used as the liquid phase.[2] Recently, Brochmann-Hanssen, *et al.*[3] reported that a number of alkaloids was successfully chromatographed on columns containing polar liquid phases such as XE-60, EGSS-X, and HI-EFF 8B.

In the work on alkaloids of *Lycoris radiata* Herb., galanthamine, lycorenine, and tazettine were separated by gas chromatography with SE-30 column[4] by Lloyd, *et al.*,[5] and separation of galanthamine, lycoramine, and tazettine[4] was done by Yamaguchi, *et al.*[6]

Thus, gas chromatographic identification of an individual or a mixture of few lycoris alkaloids seems possible. The bulb of *Lycoris radiata* Herb. contains a number of alkaloids, such as lycorine, lycoramine, galanthamine, vitattine, tazettine, haemanthamine, hippeastrine, and homolycorine, and it is desirable to establish a system of gas-liquid chromatography whereby each of these alkaloids can be identified by a single operation.

This paper presents the results of gas chromatographic study on Amaryllidaceae alkaloids, using several polar and non-polar liquid phases. The alkaloids which have a hydroxyl group except homolycorine and undulatine showed considerable tailing, especially with polar liquid phases. For the quantitative work, these alkaloids were converted into the respective trimethylsilyl (TMS) ethers.

Experimental

The TMS derivatives of alkaloids were prepared by the method of Sweeley, *et al.*[7] and chromatographed in a Shimadzu model GC-1C equipped with a hydrogen flame ionization detector. U-Shaped glass column, 1.87 m long and 4 mm in diameter, was used. The stationary liquids used were SE-30 (methylsilicone polymer), XF-1105 (cyanoethylsilicone polymer), XF-1150 (cyanoethylsilicone polymer), XE-60 (cyano-

1) Location: *16 Edagawa-cho 4-chome, Nishinomiya, Hyogo-ken.*
2) G.J. Kapadia and G.S. Rao, *J. Pharm. Sci.*, **54**, 1817 (1965); A.H. Chalmers, C.C.J. Culvenor, and L.W. Smith, *J. Chromatog.*, **20**, 270 (1965); J.L. Massingill, Jr. and J.E. Hodgkins, *Anal. Chem.*, **37**, 952 (1965); E. Schmerzler, W. Yu, M.I. Hewitt, and I.J. Greenblatt, *J. Pharm. Sci.*, **55**, 157 (1966), and references cited therein.
3) E. Brochmann-Hanssen and C.R. Fontan, *J. Chromatog.*, **19**, 276 (1965), **20**, 394 (1965).
4) In both cases, the free bases were chromatographed.
5) H.A. Lloyd, H.M. Fales, P.F. Highet, W.J.A. Vanden-Heuvel, and W.C. Wildman, *J. Am. Chem. Soc.*, **82**, 3791 (1960).
6) K. Yamaguchi, H. Ogawa, and S. Natori, *Eisei Shikensho Hokoku*, **80**, 17 (1962).
7) C.C. Sweeley, R. Bentley, M. Makita, and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

ethylmethylsilicone polymer), PEG 20M (polyethylene glycol, approximate molecular weight 20000), ECNSS-S (ethylene glycol succinate–cyanoethylsilicone polymer), EGSS-X (ethylene glycol succinate–methylsilicone polymer), NGS (neopentyl glycol succinate polymer), and HI-EFF 8B (cyclohexanedimethanol succinate polymer). The solid support was acid-washed Gas Chrom P, 60—80 mesh, which was silanized with dimethyldichlorosilane. The silanized support was coated with the stationaty liquid by the filtration method of Horning, *et al.*[8] to give a coating of 3% by weight.

The alkaloids were dissolved in anhydrous pyridine (distilled over BaO) (*ca.* 0.1 ml/1 mg). Chrysene, the commercially available aromatic hydrocarbon, was added as the internal standard. To this solution

Table I. Relative Retention Times for Alkaloid–TMS Ethers on a Variety of Stationary Phases. (1)

Alkaloid	SE-30 220°	XF-1105 220°	XE-60 220°	XF-1150 220°	ECNSS-S 210°	ECNSS-S 220°
Norpluviine	1.14	1.12	0.54	0.34		0.31
Lycorine	1.23	1.24	0.61	0.38	0.37	0.37
Lycoramine	0.97	0.97	0.60	0.43	0.40	0.42
Galanthamine	1.00	0.99	0.66	0.47	0.46	0.46
Buphanamine	1.08	1.05	0.62	0.43	0.46	0.46
Vitattine	0.93	0.91	0.58	0.44	0.52	0.53
Crinine	0.93	0.91	0.58	0.44	0.52	0.53
Tazettine	1.29	1.29	0.74	0.49	0.56	0.57
Haemanthamine	1.23	1.29	0.85	0.65	0.75	0.74
Crinamidine			1.59		1.66	
Hippeastrine	2.48	3.01	2.85	2.43	2.94	2.63
Undulatine[a]	2.00	2.12	2.09	1.96	3.19	2.90
Homolycorine[a]	2.03	2.53	3.56	4.02	5.19	4.57
Chrysene	1.00	1.00	1.00	1.00	1.00	1.00
(min)	(7.3)	(11.9)	(17.0)	(11.7)	(10.8)	(11.2)
N_2 (ml/min)	50	50	50	50	77	50

a) Free bases were chromatographed.

Table II. Relative Retention Times for Alkaloid–TMS Ethers on a Variety of Stationary Phases. (2)

Alkaloid	NGS 220°	NGS 230°	PEG-20M 220°	PEG-20M 230°	EGSS-X 220°	EGSS-X 230°	HI-EFF 8B 220°	HI-EFF 8B 240°
Norpluviine					0.21	0.21	0.20	0.21
Lycorine	0.59	0.49	0.45	0.44	0.28	0.26	0.27	0.26
Lycoramine	0.48	0.48	0.42	0.40	0.32	0.34	0.30	0.31
Galanthamine	0.54	0.53	0.47	0.45	0.42	0.39	0.36	0.35
Buphanamine	0.55	0.55	0.54	0.52	0.41	0.41	0.35	0.35
Vitattine	0.61	0.60	0.56	0.53	0.50	0.48	0.44	0.43
Crinine	0.61	0.60	0.56	0.53	0.50	0.48	0.44	0.43
Tazettine	0.73	0.71	0.67	0.63	0.52	0.50	0.49	0.47
Haemanthamine	0.87	0.83	0.79	0.70	0.69	0.64	0.62	0.58
Crinamidine		1.68				1.44		1.18
Hippeastrine	2.64	2.44	——[b]	——[b]	2.73	2.30	1.97	1.67
Undulatine[a]	2.66	2.48	2.67	2.34	3.53	3.02	2.27	1.97
Homolycorine[a]	3.41	3.13	3.52	3.00	5.10	4.35	2.80	2.35
Chrysene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(min)	(22.2)	(17.8)	(37.2)	(22.2)	(19.2)	(14.9)	(64.0)	(22.0)
N_2 (ml/min)	60	58	60	72	77	60	55	66

a) Free bases were chromatographed. b) No response.

8) E.C. Horning, E.A. Moscatelli, and C.C. Sweeley, *Chem. & Ind.* (London), 751 (1959).

were added 0.01 ml of hexamethyldisilazane and 0.01 ml of trimethylchlorosilane. After standing about 30 min at room temperature, an appropriate aliquot of the reaction mixture was directly injected into one of the columns, which was operated at 210—240°.

The relative retention values of TMS ethers of thirteen Amaryllidaceae alkaloids are given in Tables I and II, and the typical chromatograms of the mixture of TMS ethers of twelve alkaloids are shown in Figs. 1, 2, and 3.

Results and Discussion

Most of the trimethylsilyl ethers showed more symmetrical peaks and less tailing than the original alkaloids. The TMS derivative of lycorine was detected as a sharp symmetrical peak, while its non-volatile free base gave no response.

Comparison of the ability of liquid phases to separate thirteen alkaloids, as shown in Table I, indicated that SE-30 column could not separate some of the alkaloids, such as a group of vitattine-lycoramine-galanthamine, and of tazettine-haemanthamine-lycorine, and a pair of undulatine-homolycorine and norpluviine-buphanamine. With XF-1105 column (the less polar nitrile silicone), each pair of lycoramine and galanthamine, and of tazettine and haemanthamine appeared as one peak. With a more polar nitrile silicone columns (XE-60 and XF-1150), the early eluted compounds, lycorine, lycoramine, galanthamine, vitattine, buphanamine, and tazettine, were not well separated. Even with EGSS-X colunm, tazettine and vitattine were overlapped, and homolycorine had the largest relative retention value which was more than double the value obtained on a less polar column. With NGS column, lycorine and lycoramine, and with PEG 20M column, lycorine and galanthamine, were not separated, and with the latter, hippeastrine was not detected. Separation of tazettine with these two columns was inferior to that with another column (Fig. 1). Separation of these alkaloids on ECNSS-S column was satisfactory but the complete separation was not obtained for a pair of vitattine-tazettine and of hippeastrine-undulatine (Fig. 2). The HI-EFF 8B column gave an excellent separation of all these alkaloids except buphanamine which is not contained in *Lycoris radiata* Herb. (Fig. 3). No one column could separate vitattine and crinine (mirror isomer of vitattine), and buphanamine from the other alkaloids.[9] These relationships are illustrated in Fig. 5.

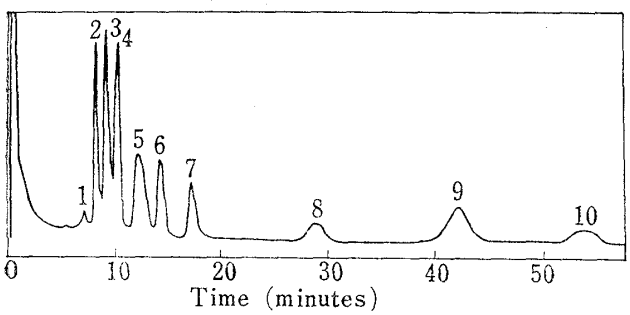

Fig. 1. Gas Chromatographic Separation of a Mixture of TMS Derivatives of Alkaloids on 3% NGS Column (230°)

The numbered peaks refer to: (1) norpluviine, (2) lycorine-lycoraminne, (3) galanthamine-buphanamine, (4) vitattine, (5) tazettine, (6) haemanthamine, (7) chrysene, (8) crinamidine (9) undulatine-hippeastrine, (10) homolycorine

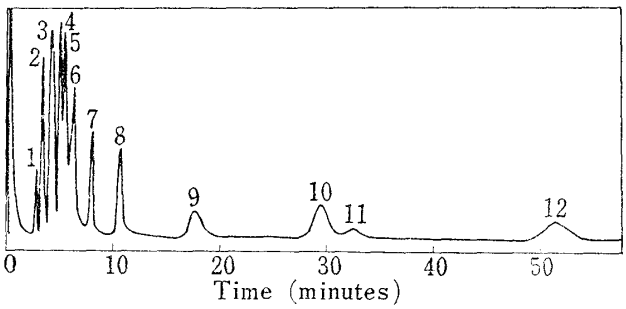

Fig. 2. Gas Chromatographic Separation of a Mixture of Alkaloid TMS Ethers on 3% ECNSS-S Column (210°)

The numbered peaks refer to: (1) norpluviine, (2) lycorine, (3) lycoramine, (4) galanthamine-buphanamine, (5) vitattine, (6) tazettine, (7) haemanthamine, (8) chrysene, (9) crinamidine, (10) hippeastrine, (11) undulatine, (12) homolycorine

9) When the mixture of free bases was put on HI-EFF 8B column operated at 240°, separation was satisfactory for buphanamine but lycorine, tazettine, hippeastrine, and norpluviine were not detected (Fig. 4).

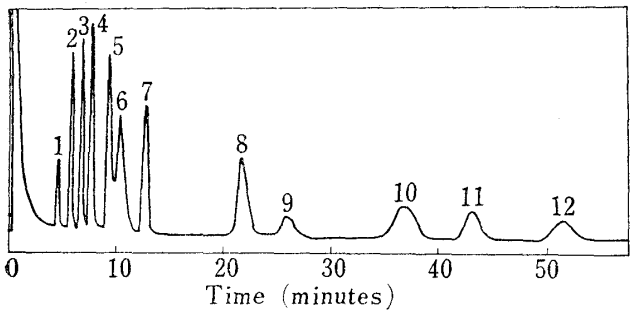

Fig. 3. Gas Chromatographic Separation of a 12-Component Mixture of Alkaloid TMS Ethers

This sample was chromatographed on 3% HI-EFF 8B column at 240°, N_2 flow rate, 66 ml/min. The components are as follows: (1) norpluviine, (2) lycorine, (3) lycoramine, (4) galanthamine-buphanamine, (5) vitattine, (6) tazettine, (7) haemanthamine, (8) chrysene, (9) crinamidine, (10) hippeastrine, (11) undulatine, (12) homolycorine.

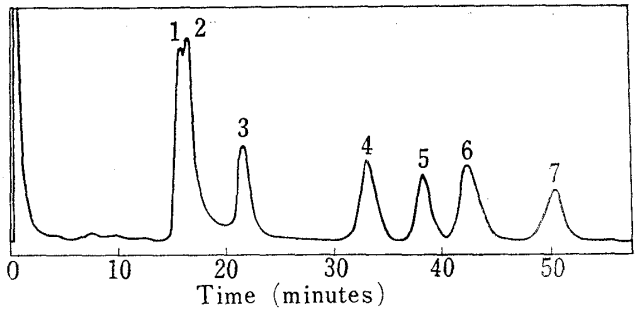

Fig. 4. Gas Chromatograms of a Mixture of Free Alkaloids on 3% HI-EFF 8B Column (240°)

(1) galanthamine, (2) lycoramine, (3) chrysene, (4) vitattine, (5) buphanamine, (6) undulatine, (7) haemanthamine-homolycorine.

These free alkaloids produced tailing peaks and lycorine, tazettine, hippeastrine, and nopluviine were not detected.

Lycorenine, one of the main alkaloids of *Lycoris radiata* Herb., showed two or three small peaks which may be due to decomposition of the original alkaloid.

Polarity of the stationary liquids affects the order of elution. For example, hippeastrine has the longest retention time with SE-30 and XF-1105 column, but this alkaloid is eluted faster than homolycorine with XF-1150 and XE-60 columns, and faster than undulatine with EGSS-X and HI-EFF 8B columns. Lycorine was eluted faster with polar column than with non-polar column. Closely related alkaloids, lycoramine and galanthamine, require an efficient column for separation. They could not be separated on a less polar column (SE-30 and XF-1105), but were readily separated on a more polar column.

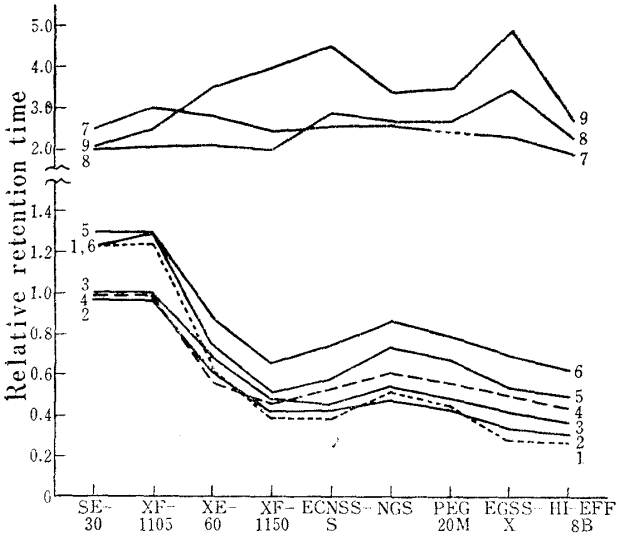

Fig. 5. Relationship between Relative Retention Time of Alkaloid TMS Ethers and Several Stationary Liquids at 220°

(1) lycorine, (2) lycoramine, (3) galanthamine, (4) vitattine, (5) tazettine, (6) haemanthamine, (7) hippeastrine, (8) undulatine, (9) homolycorine

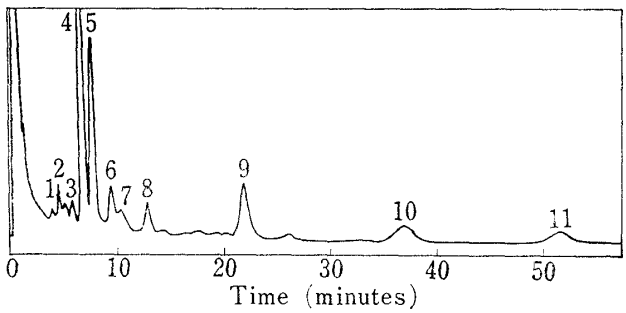

Fig. 6. Analysis of Alkaloids in the Bulb of *Lycoris radiata* Herb. as Their TMS Derivatives

The sample was chromatographed on 3% HI-EFF 8B column at 240°. The following are assignments made by comparison with chromatograms of the TMS derivatives of individual alkaloids. (1) norpluviine, (2) one of the peaks of lycorenine, (3) lycorine, (4) lycoramine, (5) galanthamine, (6) vitattine, and one of the peaks of lycorenine, (7) tazettine, (8) haemanthamine, (9) chrysene, (10) hippeastrine, (11) homolycorine

For the application of this analysis to the alkaloids of *Lycoris radiata* Herb., the crude extract obtained from the dried powder of the bulbs was separated into neutral, acidic, and basic fractions. The anhydrous pyridine solution of the basic fraction was treated with the reagent for trimethylsilanization and put on a gas chromatographic column. The typical

chromatogram is shown in Fig. 6. Several unidentified small peaks may be due to alkaloids or other substances.

Acknowledgement We are grateful to Prof. W.C. Wildman, Department of Chemistry, Iowa State University of Science and Technology, and to Dr. K. Kotera, Shionogi Research Laboratory, Shionogi & Co., Ltd., for their generous supply of the authentic samples of the alkaloids.