Notes

CHROM. 4077

A quantitative determination of cytisine and methylcytisine by gas chromatography

Cytisine[1], the main alkaloid constituent of *Cytisus laburnum (Laburnum vulgare)*, is accompanied on chromatography by the minor alkaloids methylcytisine[2] and laburnine[3], and a base, $C_{12}H_{22}NO_2$[4]. PÖHM[5] investigated the ratio of cytisine to methylcytisine in germinating seeds of this plant by quantitative paper chromatography and found that the content of methylcytisine increases considerably during the germination, an observation of some interest from the biogenetic point of view. We encountered a similar problem while investigating the alkaloid content in tissue cultures of *Cytisus laburnum* when it was necessary to estimate the cytisine to methylcytisine ratio during the growth of the culture. As paper chromatography was considered to be a time-consuming and relatively complicated method, the determination of these two alkaloids was attempted by gas chromatography. LLOYD *et al.*[6] were interested in the gas chromatography of lupine alkaloids and had carried out this separation on a 2–3% SE-30-impregnated Chromosorb W column. We ascertained that cytisine generally "tails" considerably on a 3% SE-30 column, and the use of alkaline carriers did not improve the separation. Much better results were obtained using a 5% XE-60-impregnated Chromosorb W AW column, although cytisine "tails" to some extent even with this packing.

Experimental

Apparatus. A Chrom III gas chromatograph (Laboratorní přístroje, Prague) equipped with a flame ionization detector and an all-glass system was employed.

Chromatography conditions. A glass tube column, 120 cm in length, 3 mm internal diameter, was packed with 5% XE-60-impregnated Chromosorb W AW (80–100 mesh). Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. The column temperature was 200° isothermically, and the injector temperature was 200°. A 10-μl Hamilton microsyringe was used to inject 2–3 μl of the sample.

Derivatives. Cytisine was obtained by chromatography of a mixture of raw alkaloids (extracted from seeds of *Cytisus laburnum*) on Kieselgel G (Merck) thin-layer plates in a chloroform–ethanol (8.5:1.5) solvent system. The band having an R_F value of 0.14 was scrapped off and eluted with ethanol. The cytisine thus obtained was purified by sublimation at 140°/0.1 mm Hg. The melting point was 155.5° and $[\alpha]_D$ was $-119 \pm 2°$ (H_2O).

Methylcytisine was obtained by methylation of cytisine with an equivalent amount of methyl iodide in a sealed tube at 100°, and the base was chromatographed as described above. The band having an R_F value of 0.72 was eluted with ethanol

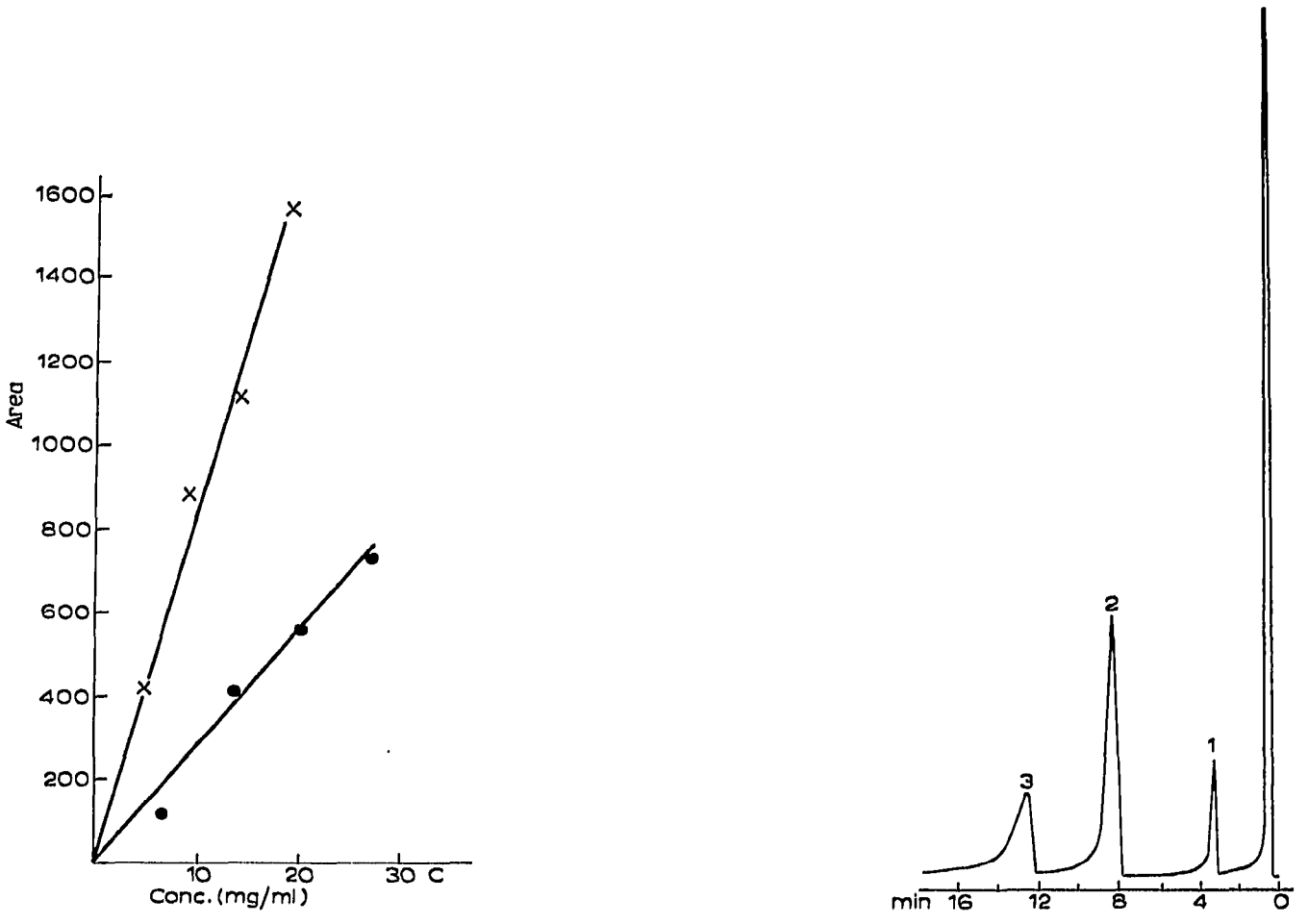

Fig. 1. Calibration curves of cytisine and methylcytisine. ×—× = methylcytisine; ●—● = cytisine.

Fig. 2. Chromatogram of the mixture of atropamine (1), cytisine (2) and methylcytisine (3).

and crystallized twice from acetone to give the pure methylcytisine. The melting point was 137° and $[\alpha]_D$ was $-222 \pm 2°$ (H_2O).

Results and discussion

The method of CONDAL-BOSCH[7] was used to calculate the peak areas in spite of the fact that this is quite laborious, since the results obtained with this method are most accurate for estimating "tailing" of the substances. Calibration curves are shown in Fig. 1.

Since contaminants were shown to cause "tailing", thereby making the evaluation difficult, the alkaloids were purified on Kieselgel thin-layer plates. Zones containing alkaloids were collected and extracted, and atropamine was added as a reference substance. This alkaloid mixture was determined quantitatively by gas chromatography. The retention times of methylcytisine and cytisine relative to that of atropamine are given in Table I.

TABLE 1

RETENTION TIMES (RELATIVE TO ATROPAMINE) OF METHYLCYTISINE AND CYTISINE

Substance	*Rel. ret. time*
Atropamine	1.00 (2.90 min)
Methylcytisine	2.64
Cytisine	4.20

A typical chromatogram of the determined substances is shown in Fig. 2.

Institute of Chemistry,
Slovak Academy of Sciences,
Bratislava (Czechoslovakia)

P. ŠEFČOVIČ
D. ANDERLE

1 A. HUSEMANN AND W. MARMÉ, *Z. Chem.*, (1865) 161.
2 M. PÖHM AND F. GALINOVSKY, *Monatsh. Chem.*, 84 (1953) 1197.
3 F. GALINOVSKY, H. GOLDBERGER AND M. PÖHM, *Monatsh. Chem.*, 80 (1949) 550.
4 F. GALINOVSKY, O. VOGL AND H. NESVADBA, *Sci. Pharm.*, 21 (1953) 256.
5 M. PÖHM, *Monatsh. Chem.*, 90 (1959) 58.
6 H. A. LLOYD, H. M. FALES, P. F. HIGHET, W. J. A. VANDENHEUVEL AND W. C. WILDMAN, *J. Am. Chem. Soc.*, 82 (1960) 3791.
7 L. CONDAL-BOSCH, *J. Chem. Educ.*, 41 (1964) A235.

Received March 17th, 1969

J. Chromatog., 42 (1969) 258–260