*Journal of Chromatography*, 281 (1983) 362–366
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,189

## Note

# Rapid quantitative analysis of lycorine by reversed-phase high-performance liquid chromatography

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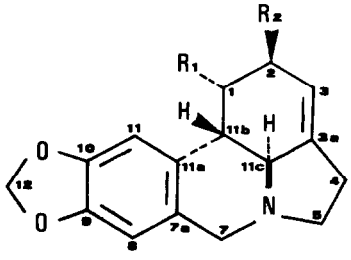
(Received August 2nd, 1983)

Interest in lycorine[1], an alkaloid extracted from various Amaryllidaceae[2], is growing because of its interesting biological activity[3–5]. No method has yet been reported for determining lycorine levels in biological samples.

Gravimetric methods[6–8] allow the determination of alkaloid yields following purification by multiple steps from dried vegetable material. However, spectrophotometric and thin-layer chromatographic methods are not suitable for analyses of crude extracts.



Lycorine $R_1 = R_2 = H$

1,2-O,O-diacetyl-lycorine $R_1 = R_2 = CH_3CO$

In this paper we report a convenient and reliable method for lycorine determination using reversed-phase high-performance liquid chromatography (RP-HPLC). Analyses are performed at alkaline pH on bonded octadecylsilane. The method has been standardized on the pure product and then extended to lycorine assay in bulbs and leaves of *Sternbergia lutea* Ker-Gawl collected during the withering period.

## MATERIALS AND METHODS

A Perkin-Elmer Series 3B liquid chromatograph, equipped with a LC-75 spectrophotometric detector at 290 nm with LC autocontrol and 10B chromatography data station, was used.

The analyses were performed on a Perkin-Elmer $C_{18}$/10 stainless-steel column

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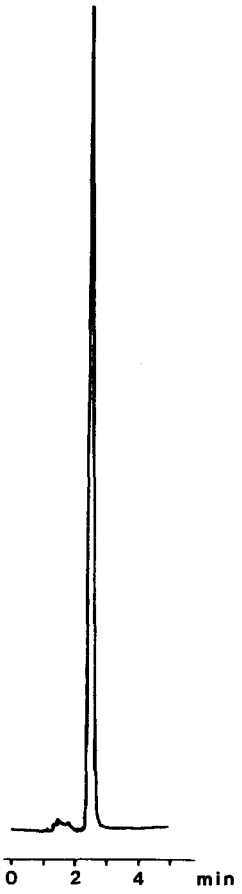


Fig. 1. High-performance liquid chromatogram of a pure sample of lycorine.

(25 cm × 4.6 mm I.D.); the mobile phase was acetonitrile (Fluka, Switzerland)–0.01 *M* ammonium carbonate (47:53, v/v) at a flow-rate of 2 ml/min. A standard solution was prepared by dissolving 10 mg of recrystallized lycorine in 100 ml of 1% sulphuric acid.

The dried and ground bulbs and leaves of the withered plant of *Sternbergia lutea* Ker-Gawl were supplied by O. Arrigoni, Istituto di Botanica, Università di Bari (Italy).

A 100-g amount of dried material (bulbs or leaves) was extracted four times with 1% sulphuric acid[9]. A portion of each extract (3 ml) was passed through a 0.2-$\mu$m membrane filter (Acrodisc, Gelman) and 20-$\mu$l portions were injected into the liquid chromatograph.

Fig. 2. Minimum detectable amount of lycorine by HPLC.

## RESULTS AND DISCUSSION

The presence in the alkaloid of the aromatic ring is responsible for its UV absorption at 240 and 290 nm ($\varepsilon$ = 3529 and 4310, respectively). Therefore lycorine can be detected by monitoring the chromatographic effluent at 290 nm; at this wavelength the non-specific interfering absorptions ($\lambda$ < 240 nm) are avoided.

The retention time of lycorine under the chromatographic conditions used was 2.55 min. A specimen chromatogram of a pure sample of lycorine is shown in Fig. 1. The detection limit for the alkaloid was 5 ng (Fig. 2). The coefficient of variation of the method obtained by six repeated analyses with two different volumes of standard solution was ± 0.5%. The average recovery determined in four independent experiments was 97.9 ± 1.7%.

The determination of lycorine in crude samples was accomplished by preparation of a calibration curve (Fig. 3). It was drawn by plotting average peak areas against concentrations of pure compound, and is linear in the concentration range 0.5–8.0 μg.

After standardization with pure sample, the method was applied to crude acid extracts of dried bulbs and leaves of *Sternbergia lutea* Ker-Gawl (Fig. 4). The presence of lycorine was confirmed by co-injection with the reference sample.

The content of lycorine in the extracts was calculated from the calibration curve by automatic peak area integration. Table I lists the results of the analysis of the samples obtained by four successive extractions with 1% sulphuric acid as described elsewhere[9]. The figures obtained for the fourth extraction demonstrate that the alkaloid was completely recovered.

The lycorine level in the extracts of the bulbs was about twice that in the leaves

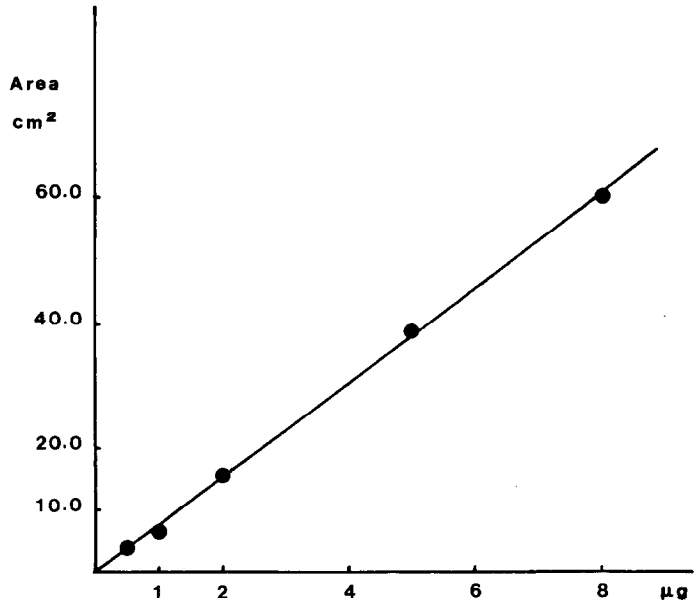


Fig. 3. Calibration curve for lycorine.

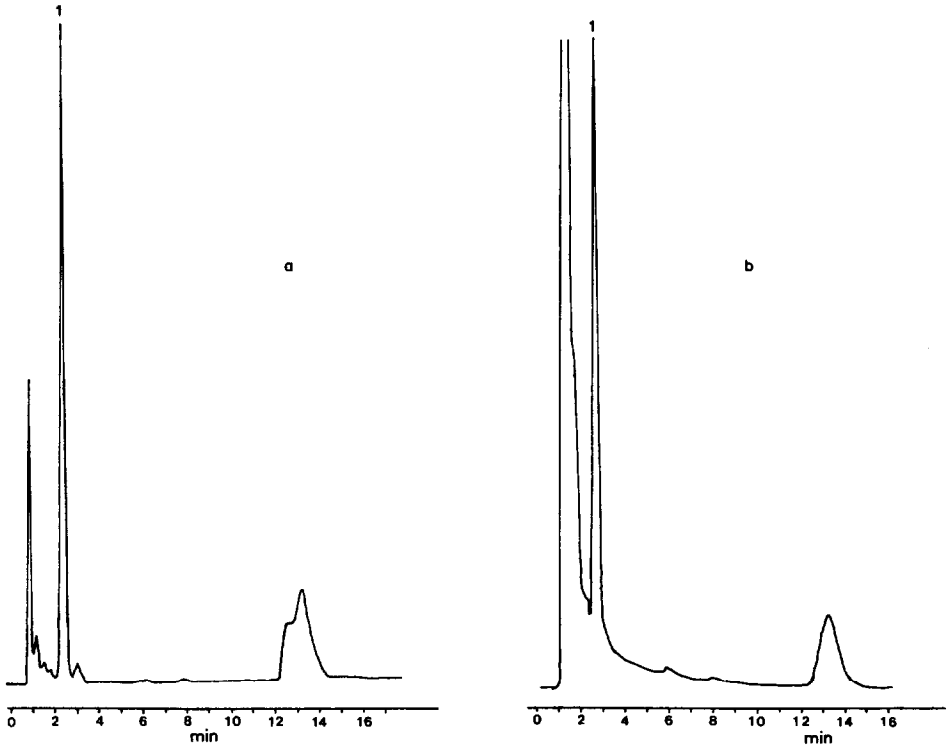


Fig. 4. High-performance liquid chromatogram of lycorine (1) in acid extracts of (a) bulbs and (b) leaves.

(2.107 and 0.910%, respectively). This result supports the hypothesis that lycorine is biosynthesized in leaves and translocated to the bulbs, where it accumulates. The alkaloid is probably translocated in a conjugated inactive form, possibly a phosphate ester, a glycoside or other derivative.

The possibility of determining lycorine in crude samples enable the estimation of yields of lycorine purification from plant material[6–9]. Reported purification procedures start with alkaloid extraction as free base. With ethanol as solvent, it has been quoted that 0.40 g (ref. 6) or 0.35 g (ref. 8) of lycorine was recovered from 100 g of dried tissue; when dichloroethane and ammonia were used, 0.03 g of lycorine

TABLE I

LEVELS OF LYCORINE IN DRIED PLANT MATERIALS OF *STERNBERGIA LUTEA* KERGAWL EXPRESSED AS WEIGHT PER CENT

| *Extract* | *Bulbs* | *Leaves* |
|---|---|---|
| First | 1.348 | 0.430 |
| Second | 0.438 | 0.289 |
| Third | 0.235 | 0.132 |
| Fourth | 0.086 | 0.059 |
| Total | 2.107 | 0.910 |

was extracted from 100 g of dried tissue[7]. According to our figures for lycorine content in dried bulbs (Table I), the yields of lycorine in these methods are 18.98, 16.61 and 1.42%, respectively.

Our purification procedure[9] involves, as the first step, alkaloid extraction as sulphate salt, then precipitation with NaOH as free base. The crude precipitate is then acetylated; the 1,2-O,O-diacetyllycorine[10] obtained is purified by silica gel chromatography. In this case, the yields of lycorine were: in bulbs, 84.575% (1.782 g for 100 g of dried material); in leaves, 36.703% (0.425 g for 100 g of dried material). The missing lycorine, as estimated by HPLC, was present in the basic mother liquors: 0.309 g in bulbs and 0.456 g in leaves (14.665 and 50.109%, respectively).

ACKNOWLEDGEMENTS

We thank Professor V. Buonocore, Istituto di Chimica Organica e Biologica, Università di Napoli, Italy, for his continued interest and helpful discussion. This research was supported by grants from the Italian Ministry of Education (Ministero della Pubblica Istruzione).

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