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## Note

# Determination of alkaloids from *Lupinus polyphyllus* by quantitative thin-layer chromatography

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A method is described for the determination of alkaloids that is simpler, more rapid and more sensitive than the formerly described procedures in which Dragendorff reagent[1] is used for detection.

Of optical methods for the direct measurement of thin-layer chromatographically (TLC) separated substances, *in situ* measurement of fluorescence gives the greatest sensitivity and reliability[2]. Röder *et al.*[3] and Messerschmidt[4] described the conversion of TLC separated alkaloids into fluorescent derivatives by spraying with dilute sulphuric acid and subsequent heating. The fluorescence depends on the number of double bonds in the molecule, and therefore this method can also be used for the determination of other organic compounds with double bonds, *e.g.*, phospholipids[5]. However, many of the lupin alkaloids, in which we are interested, are fully saturated.

Segura and Gotto[6] described a method for the detection and quantitation of several organic compounds in TLC, in which the formation of fluorescent derivatives was induced by thermal treatment of the TLC plates in the presence of hydrogen carbonate. We therefore converted lupin alkaloids, which had previously been separated by silica gel TLC, into fluorescent derivatives. This procedure permits the *in situ* determination of the alkaloids by using a fluorescence scanner.

## EXPERIMENTAL AND RESULTS

### *Extraction of the alkaloids*

The alkaloids were extracted with methanol from the leaves of *Lupinus polyphyllus*, which had previously been ground very fine by and freeze-dried. For extraction of the alkaloids we used the method of Beck[7] with slight modifications. The method of Beck is based on the repeated conversion of the alkaloids into bases and their salts.

*Production of fluorescence*

A mixture of different pure alkaloids was applied to a silica gel plate (Merck pre-coated TLC plates, silica gel 60 without fluorescent indicator, 20 × 20 cm, layer thickness 0.25 mm) and developed with benzene–dichloromethane–diethyl ether–diethylamine (5:5:5:2) in a normal saturated chamber. After development, the plates were dried and heated for 17 h at 130° in a drying chamber. When exposed to ultraviolet light of 360 nm, the alkaloids treated in this manner showed a blue fluorescence, which remained stable for several weeks. As shown in Fig. 1, the optimal fluorescence yield was achieved on heating the plate for more than 35 h. Heating at a higher temperature did not enhance the fluorescence.

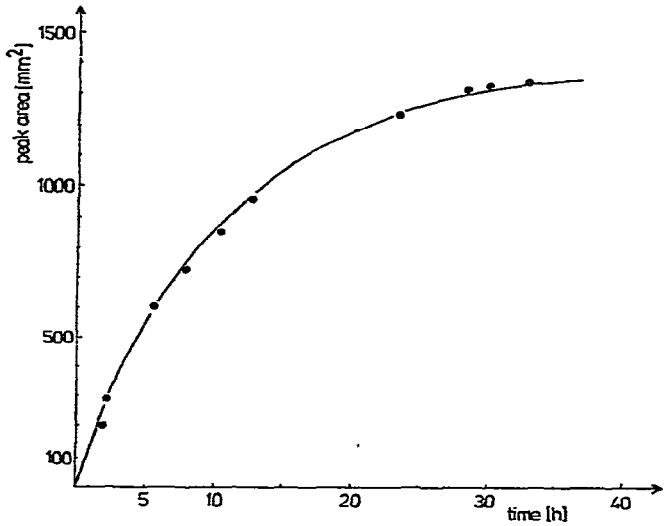


Fig. 1. Fluorescent light emitted as a function of the heating time at 130°. The curve represents the fluorescence of lupanine.

*Identification of the alkaloids in Lupinus polyphyllus*

Qualitative analysis of the alkaloids from *Lupinus polyphyllus* was carried out by two-dimensional TLC (Fig. 2). The solvent system used for the first dimension was chloroform–methanol–33% ammonia solution (95:4:1) and for the second dimension benzene–dichloromethane–diethyl ether–diethylamine (5:5:5:2). The developments were carried out in solvent-saturated chambers. The alkaloids from *Lupinus polyphyllus* (bitter type) that could be identified by comparing their $R_F$ values in the different solvent systems with those of the pure alkaloids used as references were 13-hydroxylupanine, angustifoline, lupinine, lupanine and sparteine. We are not yet able to identify the other spots shown in Fig. 2, but they seem to be alkaloids because they react with Dragendorff reagent. In this context, it should be emphasized that the reaction with Dragendorff reagent takes place even after the heating procedure.

*Quantitative evaluation of the fluorescent spots*

Different amounts of the five alkaloids identified and others in methanol were applied to a silica gel plate in form of bands by using a Linomat III apparatus (Camag,

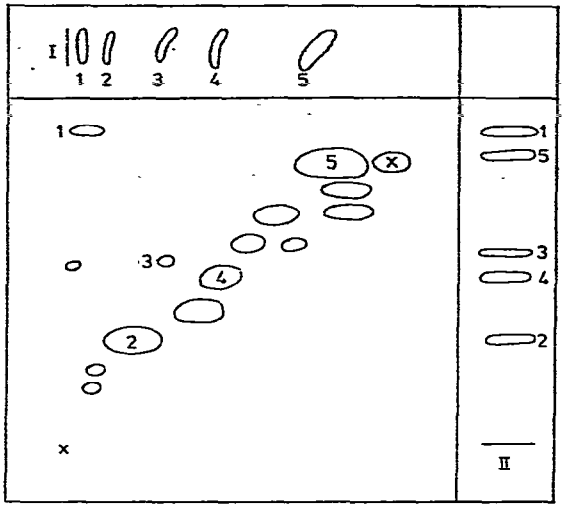


Fig. 2. Two-dimensional thin-layer chromatogram of alkaloids extracted from a bitter type of *Lupinus polyphyllus*. The conditions for the separation are described in the text. Spots: 1 = sparteine; 2 = 13-hydroxylupanine; 3 = lupinine; 4 = angustifoline; 5 = lupanine.

Muttenz, Switzerland). For separation into one row of quote we used the solvent system benzene–dichloromethane–diethyl ether–diethylamine (5:5:5:2). After drying and heating the plate for 17 h, the separated spots were evaluated by scanning the row with a Camag-T scanner. For activation, ultraviolet light was filtered through a 360-nm filter, and the fluorescence was measured at 400 nm. On plotting the peak area against the amount of alkaloids, straight lines were obtained (Fig. 3), indicating proportionality between the amount of alkaloids and the fluorescent light emitted. The lowest detection level was *ca.* 10 ng. The sensitivity of this method can be enhanced by applying the substance in the form of spots to the plate, but application in the form of bands leads to a better separation.

*Quantitative analysis of the alkaloids from bitter and sweet types of Lupinus polyphyllus*

Fig. 4 shows scanner recordings of alkaloids from a bitter and a sweet type of *Lupinus polyphyllus*. The conditions for chromatography were as described above. The number of peaks for the alkaloids from the bitter-type *Lupinus polyphyllus* was identical with the number of spots in the two-dimensional chromatogram (Fig. 2). The separation of the alkaloids was complete except for spot 5 (lupanine). As can be seen from Fig. 2, the $R_F$ value of lupanine in solvent II is identical with that of the unidentified spot X. However, separation of X can be achieved by using solvent I (see also Fig. 2). Therefore, the peak area of X could be subtracted from the peak area of (5 + X).

The quantitative evaluation of the identified alkaloids was performed by comparing the peak areas with those in Fig. 3. The results are given in Table I. It can be seen that the two types of *Lupinus polyphyllus* differ not only in the absolute amounts of the alkaloids but also in their relative composition. Strikingly, in the bitter type of *Lupinus polyphyllus* the percentage of lupanine is much higher than in the sweet type.

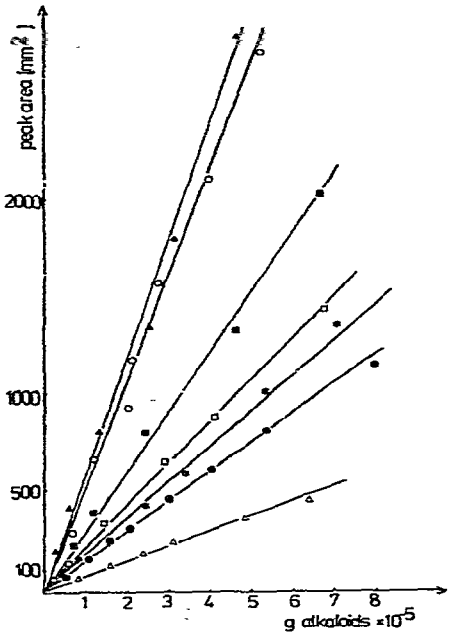


Fig. 3. Relationship between amounts of alkaloids and peak area. The conditions for chromatography and evaluation of the separated bands are described in the text. ▲, Sparteine; ○, α-isolupanine; ■, lupinine; □, 17-hydroxylupanine; ✱, lupanine; ●, 13-hydrolupanine; △, angustifoline.

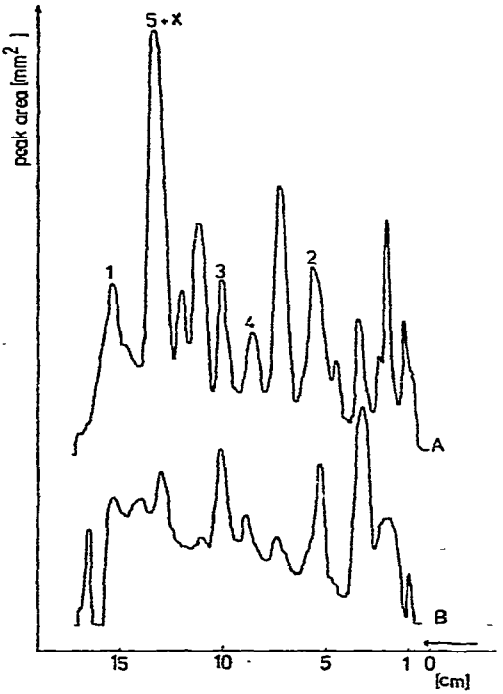


Fig. 4. Scanner recordings of the TLC plates for alkaloids of a bitter type (A) and a sweet type (B) of *Lupinus polyphyllus*; 23 times more of the sweet-type extract than of the bitter-type extract was applied to the plate. Peaks: 1 = sparteine; 2 = 13-hydroxylupanine; 3 = lupinine; 4 = angustifoline; 5 = lupanine.

TABLE I

QUANTITATIVE ANALYSIS OF ALKALOIDS FROM A BITTER TYPE AND A SWEET TYPE OF *LUPINUS POLYPHYLLUS* (SEE ALSO FIG. 4)

| *Type* | *Alkaloid (mg per g dry matter)* | | | | |
|---|---|---|---|---|---|
| | *13-Hydroxylupanine* | *Angustifoline* | *Lupinine* | *Lupanine* | *Sparteine* |
| Bitter | 3.10 | 2.30 | 0.23 | 6.11 | 0.13 |
| Sweet | 0.07 | 0.03 | 0.01 | 0.04 | 0.00 |

*Significance of the measurements*

To avoid errors caused, *e.g.*, by differences in temperature during the heating procedure, different relative humidities or differences in the materials of the plates, it is useful to carry out reference runs with pure alkaloids of known concentrations.

The TLC procedure has a good accuracy: after development of a plate with five aliquots of equal amounts of an alkaloid extract we measured a mean standard deviation of $\pm 4\%$ for the alkaloids identified.

## DISCUSSION

The classification of bitter and sweet types of lupine can be performed by a qualitative test described by Plarre and Scheidereiter[8]. The method described in this paper is applicable to the quantitative analysis of the alkaloids and is sensitive enough even for the determination of minor amounts of alkaloids from the sweet type. This analysis is important because, as shown in Fig. 3 and Table I, the two types of *Lupinus polyphyllus* differ not only in the absolute amounts of the alkaloids but also in their relative composition. Our method is simple and therefore can be recommended for routine measurements, which are necessary, *e.g.*, for screening in a breeding programme.

So far, we investigated only the alkaloid patterns in *Lupinus polyphyllus*, but there is no doubt that this method can also be used for the evaluation of alkaloids from other species of *Lupinus*.

## ACKNOWLEDGEMENTS

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