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Note

Centrifugal chromatography of diterpenoid alkaloids

Use of aluminium oxide containing two fluorescent materials

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In our efforts to develop efficient and rapid methods for the isolation of pure compounds we have reported the separation of a number of diterpenoid alkaloids using a "Chromatotron"[1]. During the separation of a mixture of 14-acetyldelcosine (I) and delsoline (II) on aluminium oxide, the separating bands were not visible at 254 nm and we had to use iodine vapors to follow the progress of the separation. Experience with the isolation and separation of alkaloids from various *Aconitum* and *Delphinium* species shows that many alkaloids present in the crude base extracts lack UV-absorbing chromophores. During chromatography on a "Chromatotron" such extracts give bands that are faintly visible or invisible at 254 nm. We report here the separations and purifications of these diterpenoid alkaloids using alumina rotors containing fluorescing materials active at long (365 nm) and short (254 nm) wavelengths. The separating alkaloid bands, which are faintly visible or totally invisible at 254 nm, can be seen as dark bands on a bluish-violet background at 365 nm. To our knowledge, the use of this type of alumina for alkaloid separations on a "Chromatotron" has not been previously reported.

EXPERIMENTAL

All separations and purifications were carried out on a "Chromatotron" Model 7924T (Harrison Research, Palo Alto, CA, U.S.A.). The rotors were coated with aluminium oxide 60 PF 254 + 366 (Type E, Cat. No. 1104-3, E. Merck, Darmstadt, F.R.G.). The adsorbent thickness was 1 mm* (prepared from a slurry of 60 g aluminium oxide and 70 ml water). The proportions of aluminium oxide and water were standardized after trying out various proportions with added binder (calcium sulphate hemihydrate, Baker TLC reagent). We found that no additional binder is necessary for 1-mm rotors using preparative grade aluminium oxide. The 1-mm rotors can be prepared conveniently by mixing the aluminium oxide and water at ambient temperature, shaking vigorously for 2 min, allowing the suspension to stand for 15 min, subsequently briefly swirling once more and then spreading the plates.

* So far only 1-mm rotors, made from the described aluminium oxide, have been used.

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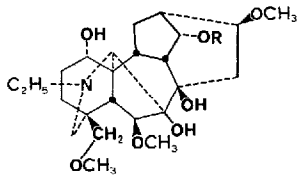
The rotor was first protected from air drafts by covering with a box for 2–3 h and then dried at ambient temperature overnight. The next day it was activated at 90–100°C for 3–4 h in an oven. The adsorbent layer on the 1 mm (scraped) rotors is stable for all non-aqueous solvents in prolonged and repeated separations. The eluting solvent was delivered by gravity feed from a reservoir kept at a height of 70 cm through the spiral inlet stopper marked – 1 (for 1-mm layers) at a rate of 2–4 ml/min. Separations were achieved using gradient elution, by a solvent system selected after trial on qualitative thin-layer chromatography (TLC) plates (aluminium oxide, 0.25 mm). Prior to the sample application, the coated rotors were washed with hexane, observing uniformity of the moving solvent front to ensure homogeneity of the adsorbent layer. An inert atmosphere was maintained by a nitrogen flow of 10–15 ml/min. The moving bands were visualized using a Spectroline® Model ENF-24 UV lamp equipped with long (365 nm) and short (254 nm) wave light (Spectronics, Westbury, NY, U.S.A.). All of the diterpenoid alkaloids reported here produced dark bands at 365 nm and were easy to detect. Occasionally the rotors were inspected in both long and short wave light simultaneously.

RESULTS AND DISCUSSION

The following separations and purifications were achieved using centrifugal chromatography ("Chromatotron") with aluminium oxide rotors containing fluorescent materials active at 254 and 365 nm.

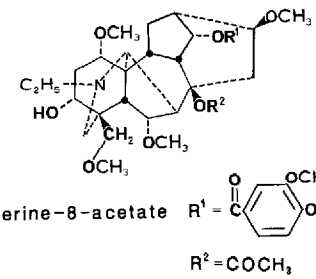
Separation of 14-acetyldelcosine (I) and delsoline (II)

A mixture of the alkaloids I and II did not show a difference in their R_F values on TLC using aluminium oxide or silica gel in most solvent systems. Diethyl ether containing 3% methanol gave a separation with an R_F difference of 0.24. The separation of I and II, using a "Chromatotron", was reported by us earlier[1] where the separated bands could not be seen in 254 nm light and iodine vapors were used to monitor their movement. Now the same mixture was easily separated on the new aluminium oxide rotor. A mixture of I (41 mg) and II (32 mg) in methylene chloride (5 ml) was applied to the alumina rotor (1 mm) and was eluted with diethyl ether containing: 0.5% methanol. Two dark bands were visible under 365 nm UV light and their movement could be followed easily. The first band was eluted quickly with diethyl ether containing 0.5% methanol and gave 39 mg of I. Since elution of the



I 14-Acetyldelcosine R=$COCH_3$

II Delsoline R=CH_3



III Falconerine-8-acetate R^1 =

$R^2 = COCH_3$

IV Ezochasmanine $R^1 = R^2 = H$

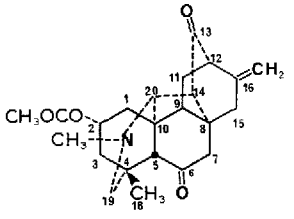
second band was slow and it had started tailing, it was eluted quickly using 0.5% diethylamine in the same solvent mixture, yielding 31 mg of II. The separation was achieved in less than 0.5 h. The eluted compounds were identified by TLC, IR and ^{13}C NMR spectra.

Isolation of ezochasmanine (IV) from a reaction mixture

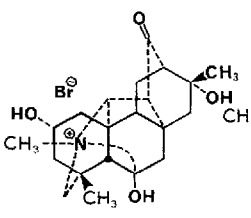
Alkaline hydrolysis of the alkaloid falconerine-8-acetate (III)[2], gave a major compound accompanied by some polar and non-polar trace impurities. In qualitative TLC the major spot of the product could not be seen easily at 254 nm or with a spray of Dragendorff's reagent. However, it gave a dark brown spot when the plate was sprayed with Wagner's reagent[3]. The reaction product (40 mg) dissolved in 2–3 ml of methylene chloride was applied to an alumina rotor and the solvent evaporated by using a larger flow of nitrogen. Elution with diethyl ether showed a major dark band, easily visible at 365 nm, moving toward the edge. This band was collected to give 16 mg of a gum which readily crystallized from benzene–hexane (1:1) to give a solid, m.p. 116–118°C, $[\alpha]_D^{21}$ 40.1° (*c* 0.35 chloroform), identical, in all respects [MMP (undepressed), IR, ^1H and ^{13}C NMR spectra] with an authentic sample of IV.

Purification of heterophylloidine (V)

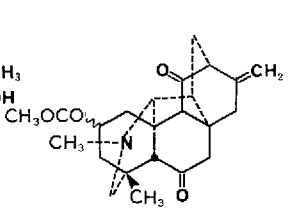
An amorphous diterpenoid alkaloid, heterophylloidine (V) ($[\alpha]_D^{24}$ − 82.0° in chloroform) was reported by us in 1981 from the Indian plant *Aconitum heterophylloides* Stapf[4]. On the basis of NMR data and an X-ray analysis of a bromine derivative (VI), the structure and absolute configuration of V was assigned. Later Katz and Staehelin[5], unaware of the publication on heterophylloidine[4], described the isolation from the European species *Aconitum paniculatum* Lam. of an alkaloid which they named "panicutin". On the basis of UV, mass spectrometric, ^1H and ^{13}C NMR data they assigned structure VII to "panicutine". This structure differs from that of V in the location of one of the keto groups at C-11 rather than C-13 and leaves the stereochemistry at C-2 undecided. However, the ^{13}C NMR spectra reported for the two compounds are nearly identical and, in fact, circular dichroism measurements have recently shown that "panicutine" is identical with heterophylloidine and has structure V[6].



V Heterophylloidine



VI



VII Panicutine

Comparison of the physical constants reported for "panicutine"[5] (m.p. 160–165°C; $[\alpha]_D^{26}$ − 141° in chloroform) with our values for heterophylloidine[4] (amorphous; $[\alpha]_D^{24}$ − 82° in chloroform) suggested that our sample might be impure.

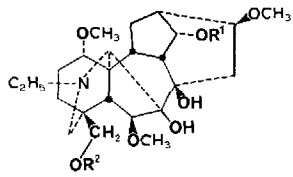
A 19-mg sample of heterophylloidine[6] was applied to an alumina rotor (1 mm) and gradient elution with methylene chloride and its mixtures with methanol was used for separation. During elution with methylene chloride three very narrow bands emerged from the center followed by a comparatively wide band faintly visible in both 254 and 365 nm UV light. The wider band exited during elution with methylene chloride containing 2% methanol and small fractions (1–2 ml) were collected during its exit. The fractions were examined by TLC and homogeneous fractions were combined to give 9 mg of a solid. The solid, when recrystallized from diethyl ether–pentane (1:1), gave white crystals, m.p. 159–161°C, identical [TLC, CO-TLC (*i.e.*, TLC which involves running an unknown on top of an authentic sample on the same plate), superimposable IR spectra] with an authentic sample of "panicutine".

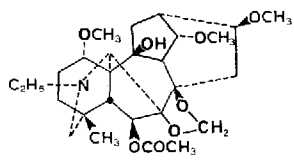
The solutions collected during elution with methylene chloride containing 2% methanol showed strong fluorescence in 365 nm light, indicating that some of the fluorescent material was washed from the rotor by these relatively polar solvents.

Purification of a sample of lycoctonine (VIII)

The C_{19}-diterpenoid alkaloid lycoctonine (VIII) has been isolated from many *Aconitum* and *Delphinium* species and various melting points have been reported for this alkaloid in the literature[7]. In our laboratory we have always encountered difficulties in the isolation and crystallization of this alkaloid. It is not easily visualized under 254 nm UV light or by spraying with the Dragendorff's reagent on a TLC plate. However, it gives a dark brown spot when sprayed with Wagner's reagent[3].

A sample of impure lycoctonine (60 mg, m.p. 95–126°C) was purified on a "Chromatotron". Gradient elution involving the solvents, hexane, methylene chloride and methanol was used. During elution three dark bands were visible under 365 nm UV light including a thin violet fluorescent band. The faintly visible major polar band (some tailing) came out during elution with methylene chloride containing 2.5% methanol. The fractions containing this major polar compound were combined to give a colorless gum (37 mg). The gum was crystallized from acetone–hexane (1:1) to give a compound, m.p. 95–97°C, identified as lycoctonine by comparison with an authentic sample (TLC, IR, ^1H and ^{13}C NMR spectra). In this experiment some elution of the fluorescent material was observed.





VIII $R^1=CH_3$; $R^2=H$ Lycoctonine

IX Deltaline

X $R^1=R^2=CH_3$ Delphatine

XI $R^1=H$; $R^2=CH_3$ Browniine

Isolation of deltaline (IX) from an alkaloid fraction from delphinium barbeyi Huth.

A fraction (107 mg) obtained by vacuum liquid chromatography (VLC[8]) of *Delphinium barbeyi* Huth. showed the presence of a major spot along with some trace

impurities on a TLC plate. The spots were not visible at 254 nm. This fraction was loaded on an alumina rotor (1 mm) and eluted with hexane–acetone (1:1). A thin band travelled with the solvent front followed by a major broad band which also came out with the same solvent. Small fractions (2–3 ml) were collected during the exit of the major band. In 10–15 min and with only 50 ml of solvent this compound (105 mg) was eluted and crystallized easily from acetone–hexane, m.p. 181.5–184°C. It was identified as IX by comparison with an authentic sample (m.p. TLC, ^{1}H and ^{13}C NMR spectra).

Isolation of delphatine (X) and browniine (XI) from another VLC fraction from Delphinium barbeyi Huth.

Another polar VLC fraction (180 mg) obtained from *D. barbeyi* Huth. showed the presence of two spots on TLC, almost invisible at 254 nm, with traces of non-polar and polar spots. The fraction was subjected to centrifugal chromatography on an alumina rotor (1 mm) and a gradient of hexane, diethyl ether and methanol was used for the elution. Two dark bands emerged from the center during elution with hexane–diethyl ether (3:17) and moved very slowly towards the edge. The first band was collected with diethyl ether containing 1% methanol to give a colorless gum (25 mg). The second band had almost stopped moving and began to tail. Diethylamine 0.2% was added to the same solvent whereupon the band sharpened but did not move. Finally it was eluted with diethyl ether containing 3 and 5% methanol and 0.3% diethylamine and was collected. The first band consisted of 25 mg of X and the second band 65 mg of XI. The compounds were identified by comparing their TLC and ^{13}C NMR spectra with those of authentic samples.

CONCLUSIONS

These experiments demonstrate the versatility of centrifugal chromatography ("Chromatotron") in the separation of complex alkaloid mixtures. The use of aluminium oxide containing two fluorescent materials improves the efficiency and reduces the time required for separations. The alumina used in these separations, Type E, is basic (pH 9) and its use should be restricted to compounds stable to base. The combination of VLC to effect preliminary separation followed by purification of these fractions on the "Chromatotron" has worked well in our laboratory. We recommend the use of gradient elution chromatography to resolve mixtures on the "Chromatotron".

As described above, some of the material fluorescing at 365 nm is washed from the alumina by solvents such as acetone and methylene chloride-methanol mixtures. This loss will reduce the sensitivity of that rotor so that it may not show good bands when small samples are used. This behavior will reduce the number of times that a given coating may be used or will restrict the use of such a rotor to the separation of large samples. However, the quantity of fluorescent material eluted is actually very small and does not seriously contaminate the samples isolated.

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