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Note

Separation of diterpenoid alkaloid mixtures using the Chromatotron

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In our efforts to develop efficient and faster methods for the isolation of pure compounds from crude mixtures of alkaloids, we report here the separation of some closely related diterpenoid alkaloids on the Chromatotron, a centrifugally accelerated, radial, thin-layer chromatography (TLC) instrument. The use of silica rotors for the separation of xanthones, triterpenes, saponins and retinoids has been documented in earlier literature[1,2], but the separation of alkaloid mixtures using silica or alumina rotors has not been reported.

The techiques of separation of complex diterpenoid alkaloids of *Aconitum*, *Delphinium* (Ranunculaceae) and *Garraya* (Garryaceae) species has been recently discussed[3]. Most of the methods used for the isolation of alkaloids in a pure state are laborious and time consuming. In connection with our work on the isolation of alkaloids from *Delphinium* and *Aconitum* species[4–6] we have evaluated the Chromatotron for its suitability for achieving rapid preparative separations.

EXPERIMENTAL

All separations were carried out on the Chromatotron, Model 7924T (Harrison Research, Palo Alto, CA, U.S.A.). The rotors were coated with a mixture of aluminium oxide 60 GF-254 neutral (type E, cat. No. 1092, E. Merck, Darmstadt, F.R.G.) and calcium sulfate–hemihydrate (Baker TLC reagent). The layer thickness was 1 mm (prepared from a slurry of 60 g aluminium oxide, 3.5 g calcium sulfate–hemihydrate and 65 ml water). The eluting solvent was delivered by gravity feed from a reservoir kept at a height of 70 cm by a flow through a 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 a qualitative TLC plate (aluminum oxide, 0.25 mm). Prior to the sample application, the coated plates (rotors) were pre-washed with hexane, observing uniformity of the moving solvent front to ensure homogeneity of the adsorbent layer. An inert atmosphere was maintained by nitrogen flow of 10–15 ml/min. The moving bands were visualized using a 254-nm UV lamp (Mineralight lamp, Model UVG-54, Ultraviolet Prod., San Gabriel, CA, U.S.A.). In some mixtures where the bands were not visible under UV, the progress of the separation was monitored by exposing the dry rotor (covered by a sheet of paper with a narrow slit) to a weak stream of iodine vapors. This was done

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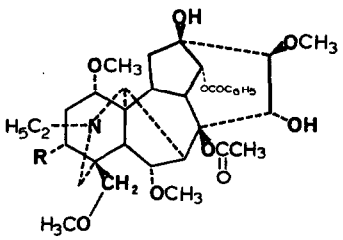
2–3 times during a separation to observe the movement of the bands. Exposure to iodine vapors was done at the same place every time by keeping a mark on the rotor, to ensure minimum loss of the alkaloids. During the separation of one of the mixtures, recycling was necessary for the resolution of the components. A reciprocating piston pump (FMI Model RP, G-150; Fluid Metering, Oysterbay, New York, NY, U.S.A.) was used for pumping the solvent mixture (2–4 ml/min).

RESULTS AND DISCUSSION

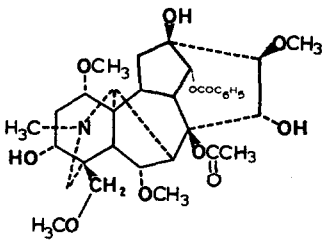
The following separations were achieved using the Chromatotron.

Isolation of alkaloids from crystalline "Aconitine Potent Merck" (Merck, Lot 30619)[3]

"Aconitine Potent Merck" is mainly a mixture of three C_{19}-diterpenoid alkaloids, *viz.*, aconitine (I), 3-deoxyaconitine (II), and mesaconitine (III). It has been



I Aconitine R=OH



III Mesaconitine

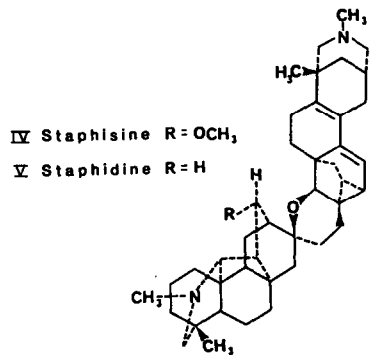
II 3-Deoxyaconitine R=H

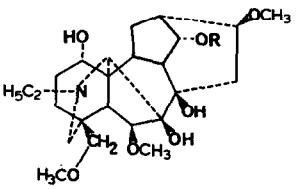
purified many times in our laboratory involving tedious and time consuming column chromatography. Mesaconitine and aconitine differ only by a methylene group at the tertiary nitrogen atom; consequently in several solvent systems the R_F values of these alkaloids are very close. A solvent system consisting of diethyl ether–methanol (100:1.9) with a run of at least 17 cm on a TLC plate gives the best separation (R_F values: aconitine 0.44, mesaconitine 0.26 and 3-deoxyaconitine 0.79). To separate this mixture on the Chromatotron, gradient elution with hexane, hexane–diethyl ether, diethyl ether and diethyl ether–methanol was used. The sample (250 mg) in methylene chloride (5 ml) was applied, the rotor dried under nitrogen and then eluted with the solvent system given above. Development with hexane–diethyl ether (25:75) (50 ml) gave in fraction 4, 9 mg of 3-deoxyaconitine, diethyl ether–methanol (99.9:0.1) (100 ml) gave in fractions 6–13, 190 mg of aconitine and 0.7 to 1.5% methanol in diethyl ether (225 ml) gave in fractions 20–28, 4 mg of mesaconitine. Recovery of the sample from all the fractions, including washing of the rotor with methanol, was complete. The total solvent used was about 1 l and the time taken was about 2 h. The alkaloids isolated were identified by comparison of their m.p., mixture m.p., specific rotation, IR, ^1H and ^{13}C NMR spectra with those of authentic samples.

Separation of two bis-diterpenoid alkaloids from "staphisine"[3]

Jacobs and Craig[7] isolated a crystalline alkaloid named "staphisine" from the

mother liquors accumulated during the isolation of delphinine from the seeds of *Delphinium staphisagria* L. On the basis of chemical studies they postulated that "staphisine" is a dimeric C_{20}-diterpenoid alkaloid. Later work on these mother liquors led to the isolation and structure determination of eight dimeric diterpenoid alkaloids[8]. The crystalline "staphisine" sample was shown to be a mixture of two bis-diterpenoid alkaloids, *viz.* staphisine (IV) and staphidine (V)[9]. Although "staphisine" gives a single spot on TLC in various solvent systems, it can be separated to give compounds IV and V by preparative TLC using gradient multiple developments[3]. The separation is a very difficult one, for the difference between these two large molecules is a single methoxyl group. As many trials failed to resolve the "staphisine" alkaloids, we resorted to recycling the eluates on the Chromatotron with the use of a reciprocating pump. A solution of the "staphisine" mixture (93 mg) was applied and after taking due care to minimize the loss of solvent, the rotor was eluted with hexane containing 4.5% acetone. After 2 recycles, a separation of the two bands was achieved and these gave 31.5 mg of staphidine and 12.5 mg of staphisine. This separation was completed using approximately 200 ml of eluent in about 2.5 h and the purity of the alkaloids isolated was checked by their ^{1}H NMR spectra. Staphisine: δ 3.30 (OCH_3) and 2.27, 2.13 (2 NCH_3) ppm; staphidine: δ 2.21, 2.13 (2 NCH_3) ppm[8].





VI Delsoline R=CH_3

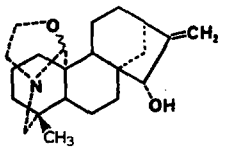
VII 14-Acetyldelcosine R=$COCH_3$

Separation of delsoline (VI) and 14-acetyldelcosine (VII)

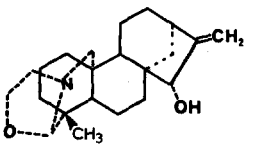
These alkaloids were reported by us earlier and their isolation involved lengthy procedures of column chromatography and preparative TLC[10]. A mixture of the alkaloids did not show a difference in their R_F values on TLC using aluminium oxide or silica gel in most solvent systems, but diethyl ether containing 3% methanol showed a separation with an R_F difference of 0.24. A 1:1 mixture of compounds VI and VII (90 mg) in methylene chloride (5 ml) was applied to an alumina rotor (1 mm) and was eluted with diethyl ether and increasing percentages of methanol. As the bands were not visible under UV light, the movement of the bands was noted by exposing the rotor (covered by a sheet of paper having a narrow slit) to iodine vapors (see Experimental). In 1.5 h 42.5 mg of compound VII was eluted in fractions 7–17 (diethyl ether–methanol, 99.5:0.5) and 36.5 mg of compound VI was isolated from fractions 20–29 (diethyl ether–methanol, 99:1). The identity of the isolated compounds was confirmed by their ^{13}C NMR spectra.

Separation of the C_{20}-diterpenoid alkaloids, veatchine (VIII) and garryine (IX)

Certain of the C_{20}-diterpenoid alkaloids are known to undergo isomerization, addition reactions and structural transformations during their isolation[11,12]. To determine whether the "normal"-oxazolidine-"iso"-oxazolidine pair can be resolved, we carried out the separation of a mixture of veatchine and garryine on the Chromatotron.



VIII Veatchine



IX Garryine

A mixture of veatchine (45 mg) and garryine (50 mg) was applied to the alumina rotor (1 mm) in dichloromethane. Elution with diethyl ether containing 1% methanol gave garryine (50 mg) and nothing further was eluted even with diethyl ether–methanol (90:10). When the rotor was washed with methanol, all the veatchine was eluted. Because of the high pK_a (11.5) of veatchine, it probably formed a salt with some residual acid in the neutral alumina used, for the eluted product was water soluble. Veatchine was regenerated from the salt by basification and extraction with methylene chloride to give 36 mg. The identity of the isolated compounds was confirmed by their ^{13}C NMR spectra.

CONCLUSIONS

The Chromatotron is a useful instrument for the separation of diterpenoid alkaloids. It is a rapid method for preparative isolations; about 250 mg can be separated on a 1-mm rotor. The samples that are loaded can be quantitatively recovered. Preparation of alumina rotors is somewhat difficult but can be achieved with practice. Resolution of the bands can be increased by using the recycling technique. For air- and light-sensitive compounds, an inert atmosphere and darkness can be maintained. The most important factor in the use of this instrument is the selection of a suitable solvent system. Gradient elution appears to be very effective and once the components are resolved on the rotor, polarity of the developing solvent should be increased rapidly taking care not to merge the separated bands. Once the conditions for separation of a particular mixture have been worked out, the separation is reproducible.

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