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SEPARATION OF DITERPENOID ALKALOID MIXTURES USING VACUUM LIQUID CHROMATOGRAPHY

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ABSTRACT.—Vacuum liquid chromatography (vlc) is an efficient, inexpensive chromatographic technique that can be used for the separation of complex alkaloid mixtures. Its application to the separation of eight mixtures of closely related diterpenoid alkaloids in both large and small samples is described. Comparison of vlc and preparative tlc for the separation of a 1.000 g commercial sample of Merck "aconitine" mixture demonstrates the superiority of the vlc method. Vlc has proved useful for the separation of mixtures of naturally-occurring alkaloids as well as mixtures resulting from synthetic operations.

In our continuing (1) efforts to separate both large and small quantities of alkaloid mixtures efficiently, rapidly, and inexpensively, we report here the separation of some closely related diterpenoid alkaloids using the vacuum liquid chromatography technique (vlc). The use of vlc was first mentioned by Coll *et al.* (2,3) for the separation of diterpenes, but no experimental details describing the method were reported. Subsequently, Targett *et al.* (4) described an elaborate set up for vlc and demonstrated its effectiveness for the separation of a standard dye mixture and a mixture of (+)-fenchone and (+)-camphor. Recently, Targett has greatly simplified the vlc apparatus with no loss of separation efficiency.[1] We report here our use of this simplified vlc technique for separation of several alkaloid mixtures.

Numerous studies of the chromatographic separation of complex diterpenoid alkaloids of *Delphinium, Aconitum,* and *Garrya* species have been reported (5). Most of the methods employed for the isolation of the alkaloids in the pure state are tedious, expensive, or are useful only for small-scale separations. Vlc has proved useful in our hands for the separation of mixtures of natural products as well as mixtures resulting from synthetic operations.

THE TECHNIQUE

The apparatus (Figure 1) consisted of a sintered-glass Büchner filter funnel with fritted disk (ASTM 10-15μ or 10-20μ; e.g., Ace Glass 7184 Filter Funnel, Porosity D) and a t 24/40 joint into which a layer of tlc grade aluminium oxide or silica gel was packed. An alternate set up involved use of a Büchner filter funnel (e.g., Ace Glass 7186 Filter Funnel) fitted with a rubber stopper. A three-way stopcock was used to control the vacuum provided by a water aspirator (20-70 mm Hg). The adsorbent was first loaded into the sintered-glass funnel and was allowed to settle by gentle tapping under gravity. Then the vacuum was applied, and the adsorbent was compressed to a hard layer by pressing with a rubber stopper and tapping. After the uniform and tight packing of the adsorbent, the vacuum was released, solvent of low polarity was poured quickly onto the surface of the adsorbent, and then vacuum was reapplied. The solvent should pass through the column uniformly. If it does not, the column must be repacked. The column was then sucked dry, and the alkaloid mixture, in the least polar solvent in which the extract or mixture can be solubilized, was carefully introduced onto the surface of the packing (no vacuum). Enough solvent was used to completely cover the top surface of the adsorbent. Then vacuum was applied gently to draw the

[1]Private communications from Dr. N.M. Targett, September 30, 1983 and February 14, 1984.

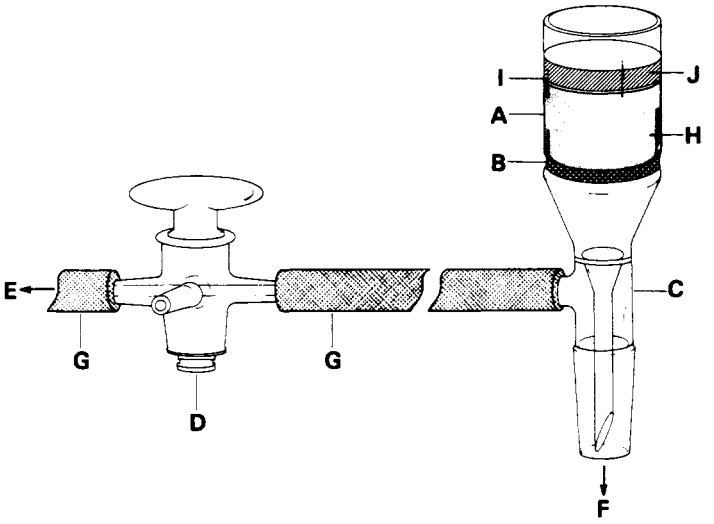


FIGURE 1. Laboratory vlc apparatus

A—Sintered glass Buchner filter funnel with fritted disk (**B**) (ASTM 10-20μ) and adapter with s 24/40 joint (**C**).
D—Three-way stopcock.
E—To vacuum (water aspirator, 15-25 mm Hg).
F—To round-bottom flask or separatory funnel.
G—Rubber tubing.
H—Adsorbent (E. Merck, tlc grade).
I—Substrate, after absorption on support.
J—Solvent.

sample into the packing. A thin, uniform line of substrate should result at the top of the column. The substrate was sometimes applied by preadsorbing on a suitable adsorbent (i.e. Al_2O_3, SiO_2 or Celite), and the latter was applied as a uniform layer at the top of the column. Both methods worked equally well. The column was then developed under gentle vacuum with appropriate solvent mixtures, pulling the column dry between each fraction collected. CAUTION: Use only a gentle vacuum (20-70 mm Hg) to avoid boiling solvents or possible collapse of glassware.

The fractions can be collected in a round-bottomed flask or in a suitable separatory funnel. The use of a separatory funnel avoids the problem of changing the flask for each fraction and also is useful for collecting very small fractions without difficulty.

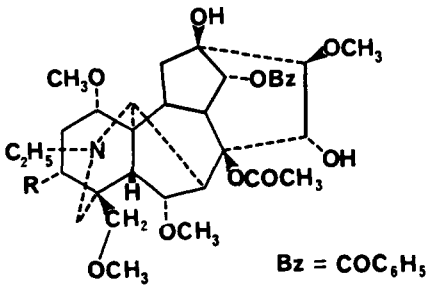
After each fraction was collected, an appropriate solvent was added to the top of the column without vacuum until the surface was well covered. Then vacuum was gently reapplied. Gradient elution was used. Channeling was easily avoided by sucking the column dry each time a fraction was changed. Resolution can be improved by increasing the ratio of support to substrate. Typical ratios used were from 30:1 to 300:1. Loss of very volatile solvents was minimized by increasing the flow rate of the solvent mixture using a larger pore size of fritted disk (ATSM 10-20μ) funnel. The adsorbents used were E. Merck products: aluminum oxide 60 H basic (type E, EM 1085, 10 μm average particle size, 60Å mean pore diameter) and silica gel 60 HR (EM 7744, 60Å).

RESULTS AND DISCUSSION

This paper describes the separation by vlc of eight mixtures of various diterpenoid alkaloids. One of these is a commercial mixture, one is a mixture of alkaloids from a plant extract, two are synthetic mixtures of alkaloids, and four are mixtures of alkaloids

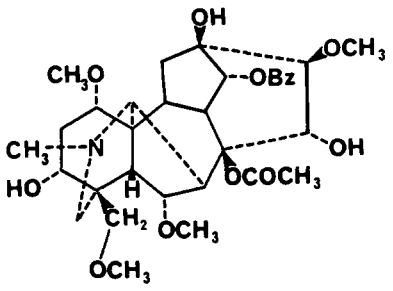
formed as reaction products. In all cases vlc proved to be a rapid and efficient method of separation.

The first separation to be described is that of commercial Merck "aconitine," a complex mixture of several closely related alkaloids, viz. aconitine (**1**), deoxyaconitine (**2**), mesaconitine (**3**), and other polar materials. This mixture is very difficult to separate by conventional column chromatography. Aconitine is expensive and extremely difficult to obtain in a pure state by gravity-column chromatography or by recrystallization of its salts. Vlc separations of Merck "aconitine" were carried out to provide substantial quantities of pure aconitine for subsequent microbial oxidation studies and other synthetic transformations.



Bz = COC_6H_5

1 aconitine R=OH
2 3-deoxyaconitine R=H



3 mesaconitine

A 1.000-g sample of Merck "aconitine" when applied to 90.0 g tlc grade Al_2O_3 in a vlc column and developed by gradient elution afforded 57 mg of 3-deoxyaconitine (**2**, Rf 0.85), 911 mg of aconitine (**1**, Rf 0.56), and 15 mg of slightly impure mesaconitine (**3**, Rf 0.47). Note that the only difference between aconitine and mesaconitine is a methylene group at the tertiary nitrogen atom.

For comparison purposes 1.000 g samples of Merck "aconitine" were separated by vlc and by preparative tlc plates. Table 1 summarizes differences for the two methods in cost of adsorbent, set-up time, fractionation time, amount of solvents consumed, time for the complete separation, resolution, and various other points.

Preparative tlc (ptlc) was very troublesome to use for separating 1.0 g of Merck "aconitine" because of the difficulty of preparing thick, uniform plates. Several experiments were necessary before separation could be achieved on the four plates used. The preparation time was about 48 h because commercial plates of the required thickness (2.5 mm) are not available. For a ptlc separation a solvent system is chosen on the basis of the qualitative tlc results for a particular mixture. We have observed that considerable loss in recovery of alkaloids often results from ptlc separation for several reasons: (a) irreversible binding of substrate to adsorbent (encountered frequently with polyhydroxy alkaloids), (b) decomposition of the compounds on the plate, (c) poor elution of substrate mixture from the origin by the solvent system selected on the basis of tlc results, (d) the use of protic solvents to develop the plates in an ascending mode prolongs elution time and increases the chances for structural changes of alkaloids on the adsorbent, e.g., rearrangement of C_{20}-diterpenoid alkaloids from "normal" to iso-forms" in the presence of alcohols (6).

The total time for the ptlc was 54 h compared with 7 h for vlc. The cost of support used in ptlc was $64, compared with $6 for the vlc method. Moreover, the vlc column can be used several times before repacking. The support on the ptlc plates once developed and extracted from the bands is not readily reusable. Also the yields of purified products from the vlc column were slightly better than those recovered from the ptlc plates: deoxyaconitine (57 vs 35 mg), aconitine (911 vs 892 mg), and mesaconitine (15

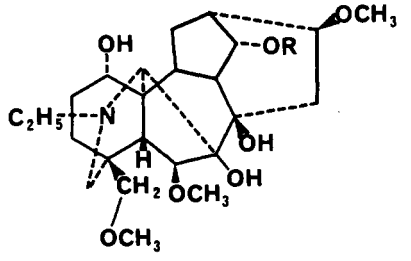
TABLE 1. Comparison of Results of vlc and ptlc Separation of 1-g Sample of Merck "Aconitine" Mixture

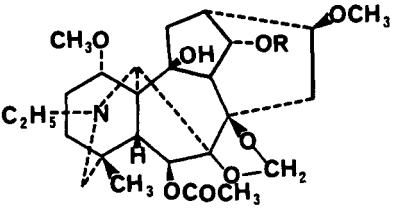
Points of Interest	vlc	ptlc
1. Al_2O_3 used; cost	90.0 g; $6.00	760.0 g; $64.00
2. Set-up time	1 h	48 h
3. Fractionation time	5 h	2 h (development) 3 h (band extraction)
4. Solvents used	1.5 liters	4.25 liters
5. Total time for the whole operation	7 h	54 h (including preparation time)
6. Resolution	Good even for large samples	Good only for small samples
7. Reusability of the support	Yes	No
8. Convenience of procedure	Easy, with readily available common lab equipment.	Requires special alumina plates which must be carefully prepared.

vs 16 mg). Overall, in our hands, we have found the vlc method is much preferable to the ptlc method for separating these alkaloids.

Sastry and Waller (7) were the first to detect the presence of delcosine (**4**), 14-acetyldelcosine (**5**), and delsoline (**6**) along with a few other alkaloids in *Consolida ambigua* L.P.W. Ball and V.H. Heywood (formerly known as *Delphinium ajacis* L.). Delcosine, 14-acetyldelcosine, and delsoline differ in structure only at C(14). In early work (8), the above three alkaloids were isolated by time-consuming gravity column and ptlc. A vlc separation was carried out to furnish a sample of 14-acetyldelcosine required for the synthesis of delcosine-7-*O*-methylether (9). Isolation of the above three alkaloids was achieved from the weak-base fraction of *C. ambigua* using the vlc method in a single pass. Thus, 500 mg of this weak base fraction was separated on a vlc column, affording 208 mg of 14-acetyldelcosine (**5**), 43 mg of delsoline (**6**), and 46 mg of delcosine (**4**).

To determine the stability of the diterpenoid alkaloids on the vlc support and the percentage of recovery, we carried out the separation of a mixture of deltaline (**7**) and 14-acetyldictyocarpine (**8**), differing only in substitution at C(14). These alkaloids





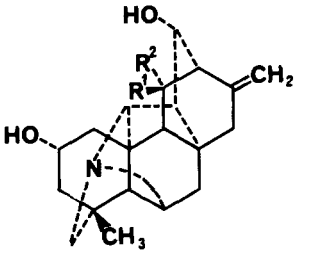
4 delcosine R=H
5 14-acetyldelcosine R=$COCH_3$
6 delsoline R=CH_3

7 deltaline R=CH_3
8 14-acetyldictyocarpine R=$COCH_3$

were reported by us earlier, and their isolation involved lengthy procedures of column chromatography and ptlc (10). Application of a mixture of detaline and 14-acetyldictyocarpine (15 mg each) to a vlc column of Al_2O_3 and development with hexane/$CHCl_3$ mixtures gave 15 mg of detaline and 14.5 mg of 14-acetyldictyocarpine. The whole experiment was completed in 1 h using only 100 ml of solvent mixture.

In our experience isolation of C_{20}-diterpenoid alkaloids using gravity column chromatography is difficult, slow, and results in the loss of the alkaloid material be-

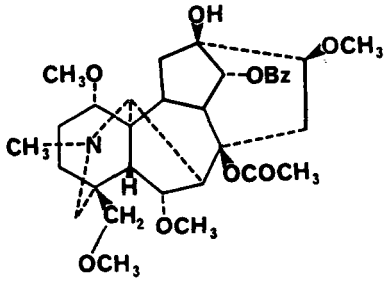
cause of decomposition, and binding to the column adsorbent (11). To determine the efficiency of the vlc method for C_{20}-diterpenoid alkaloids we carried out the separation of a mixture of 16 mg each of hetisine (**9**) and hetisinone (**10**) over Al_2O_3. Recovery was excellent with 16 mg of hetisinone and 15.5 mg of hetisine being isolated. The separation required 2 h using only 200 ml of solvents.



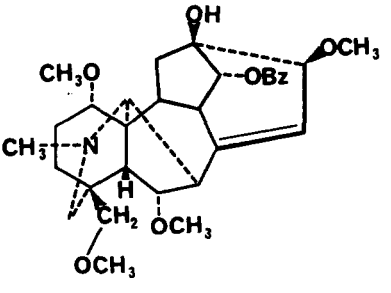
9 hetisine R^1=H, R^2=OH
10 hetisinone R^1,R^2=O

Pyrodelphinine (**12**) is a key intermediate for certain synthetic transformations and a subject of a recent study of its chromophore (12). Pyrolysis of a sample of delphinine (**11**) under reduced pressure leads to elimination of HOAc and formation of pyrodelphinine. The product mixture usually consists of a mixture of pyrodelphinine and delphinine that requires separation. This separation can be smoothly effected using a vlc column. Thus, pyrolysis of 1.25 g of delphinine afforded 1.156 g of product mixture. Separation on a vlc column of silica gel gave 896 mg of pyrodelphinine and 108 mg of delphinine and was completed in 3 h.

18-Methoxygadesine (**13**) is a rare naturally-occurring alkaloid that was isolated by Gonzalez *et al.* (13) from *Consolida orientalis* Gay; its structure was established by X-ray analysis. This carbinolamine ether alkaloid was isolated as a major product of the reaction of delcosine with OsO_4. Treatment of 64 mg of delcosine with OsO_4 gave a product mixture from which 18-methoxygadesine (34 mg) was isolated easily using a vlc column of Al_2O_3.



11 delphinine



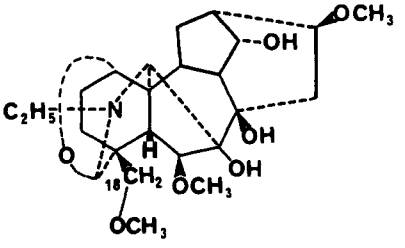
12 pyrodelphinine

Recent interest in 8-*O*-alkyl C_{19}-diterpenoid alkaloids (14,15) has led us to study the chemistry of these alkaloids. Acoforestinine (**14**)[2] was prepared from yunaconitine (81 mg) by heating it with absolute EtOH in a sealed tube at 130-135° for 24 h. The facile conversion probably proceeds through a synchronous Grob fragmentation reaction (16). Although tlc analysis indicated the product was a mixture of six compounds,

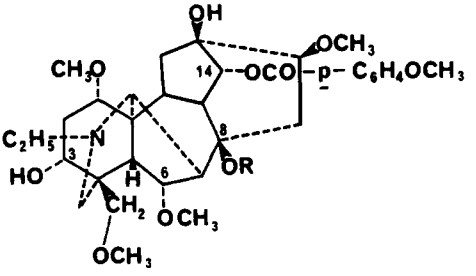
[2]B.S. Joshi, J.A. Glinski, H.D. Chokshi, and S.W. Pelletier, unpublished work.

separation on a vlc column of Al_2O_3 proceeded smoothly to give 67 mg of pure acoforestinine plus small amounts of unidentified materials.

Similarly, 8-*O*-propyl-14-anisoylyunaconine (**16**) was prepared from yunaconitine (**15**) (30.0 mg) by reaction with dry *n*-PrOH in a sealed tube at 135° for 24 h. The complex product mixture (33 mg) was resolved on a vlc column of Al_2O_3 to afford pure 8-*O*-propyl-14-anisoylyunaconine (13 mg) and unreacted yunaconitine (11 mg).



13 18-methoxygadesine



14 acoforestinine R=CH_2CH_3
15 yunaconitine R=$COCH_3$
16 8-*O*-propyl-14-anisoylyunaconine R=$CH_2CH_2CH_3$

CONCLUSIONS

Vlc is an extraordinarily simple technique, consumes small amounts of solvents, has reasonable resolution, and requires relatively little time to carry out a separation (5). The samples that are loaded can be recovered in good yield. The vlc method can be used for the separation of relatively large as well as small amounts of mixtures. Gradient elution is very effective, and the selection of a suitable solvent system is achieved by analytical tlc examinations. The vlc method uses a simple and inexpensive equipment set up, which can be assembled in any laboratory (Figure 1). The packed funnel can be reused after eluting with MeOH and lightly scraping away any decomposed polar material from the top surface of the adsorbent. In our hands we have found vlc to be superior to ptlc as well as flash chromatography (17) or dry column chromatography (18) when applied to the separation of complex diterpenoid alkaloids. Better resolution of substrates is achieved in less time as compared with conventional column chromatography.

The excellent separation possible by this method is due to the fine particle size (10 μm average), the very large surface area (500 m^2/g) of the particles, and to the method of packing (see Experimental section). The use of tlc grade support containing a binder provides a compact column which readily accommodates changes in pressure and normal handling during a separation. The separation is economical in terms of support, solvents used (see Table 1), and in the time of execution.

EXPERIMENTAL

Melting points are corrected and were taken on a Thomas-Kofler hot stage equipped with a microscope and polarizer. The rotation was taken on a Perkin-Elmer polarimeter, Model 141. Ir spectra were recorded on a Perkin-Elmer Model 1420 spectrometer. ^1H- and ^{13}C-nmr spectra were taken on a JEOL FT model FX-90Q spectrometer in $CDCl_3$ solutions with TMS as an internal reference.

ISOLATION OF ALKALOIDS FROM CRYSTALLINE MERCK "ACONITINE" (MERCK & CO., LOT 30619) BY VLC.—A solution of 1.000 g of Merck "aconitine" in 7 ml of CH_2Cl_2 was applied uniformly to the top of a vlc funnel (5×20 cm) prepared by packing with tlc grade Al_2O_3 (90 g). Gradient elution with hexane, Et_2O, and MeOH was carried out, collecting 100 ml fractions. Monitoring of fractions was done on alumina plates developed with EtOH-1.5% MeOH. Elution with hexane, hexane-Et_2O (3:1), and hexane-Et_2O (1:1) gave in fractions 5 and 6: 57 mg of 3-deoxyaconitine (**2**) (Rf 0.85); in fractions 7 and 8: 18 mg of a mixture. Elution with hexane-Et_2O (1:3) gave in fractions 9 and 10: 911 mg of aconitine (**1**) (Rf 0.56); in

fraction 11: 15 mg of a mixture. Elution with hexane-Et_2O (1:9) and Et_2O gave in fractions 12 and 13: 15 mg of mesaconitine (**3**) (Rf 0.47). The mesaconitine (15 mg) from fractions 12-13 (combined according to tlc behavior and ^{1}H-nmr spectrum) was further purified by crystallization to yield 12 mg of pure mesaconitine. Elution with Et_2O-MeOH (9:1) and (1:1) gave 29 mg of polar compounds. This separation was finished in 7 h.

The alkaloids isolated were recrystallized and identified by comparison of their mp, hplc analysis, and ^{13}C-nmr spectra with those of authentic samples. Aconitine (**1**): mp 200-204° (recrystallized from MeOH), [lit. (19) mp 202-205°]. Deoxyaconitine (**2**): mp 174.5-176° (recrystallized from EtOAc/hexane), [lit. (19) mp 177-180°]. Mesaconitine (**3**): mp 200-201° (recrystallized from Me_2CO), [lit. (20) mp 197-199°].

ISOLATION OF ALKALOIDS FROM CRYSTALLINE MERCK "ACONITINE" (MERCK & CO., LOT 30619) BY PTLC.—A 1.000-g sample of Merck "aconitine" was uniformly applied, as a thin streak, using an applicator (Kontes, Scientific Glassware/Instruments, Vineland, New Jersey), to four 20×40 cm Al_2O_3 60 PF254 (Type E, EM 1103-3, 2.5 mm thick) plates in a $CHCl_3$ solution. The plates were developed in Et_2O-1.5% MeOH (1.75 liters) in the same tank. Four bands were marked (under 254 nm) and then scraped from the plate. The scraping from each band was extracted (2-3 times), by stirring with $CHCl_3$. Band 1 (least polar) gave a gum (35 mg) which on recrystallizations from EtOAc/hexane gave 21 mg of deoxyaconitine (**2**), mp 174-176°. Band 2 (showing two close compounds) when extracted with $CHCl_3$ gave a gum (10 mg). Band 3 (major broad band) when extracted with $CHCl_3$ gave a gum (892 mg) that crystallized from MeOH to give 613 mg of aconitine (**1**), 199-202.5°. Band 4 when extracted with $CHCl_3$ gave 16 mg of mesaconitine (single spot) which crystallized from Me_2CO to give 9 mg of mesaconitine (**3**), mp 198-201°.

All the compounds were identified by comparing their physical and spectral data with those of authentic samples.

ISOLATION OF ALKALOIDS FROM *C. AMBIGUA*.—When 500 mg of the weak-base fraction was applied to a vlc column (60 ml size) containing 50 g of Al_2O_3, elution with toluene plus 40% $CHCl_3$ afforded 208 mg of 14-acetyldelcosine (**5**) in fractions 10 to 12 (125 ml each fraction). Elution with toluene plus 60% $CHCl_3$ afforded 43 mg of pure delsoline (**6**) in fractions 17-18. Finally elution with $CHCl_3$ and $CHCl_3$ plus 1.0% MeOH gave 46 mg of delcosine (**4**). The above experiment was completed in 8 h.

The alkaloids isolated were recrystallized and identified by comparison of their mp, mmp, tlc and co-tlc behavior, and ir spectra with those of authentic samples. 14-Acetyldelcosine (**5**): mp 193-195° (recrystallized from Et_2O), [lit. (19) mp 193-195°]. Delsoline (**6**): mp 212-213° (recrystallized from MeOH), [lit. (19) mp 215-216°]. Delcosine (**4**): mp 201-203° (recrystallized from Me_2CO), [lit. (19) mp 203-204°].

SEPARATION OF DELTALINE (**7**) AND 14-ACETYLDICTYOCARPINE (**8**).—A mixture of deltaline (15 mg) and 14-acetyldictyocarpine (15 mg) was applied to a vlc column (15 ml size) containing 5 g of Al_2O_3. Elution was carried out with a hexane/$CHCl_3$ mixture, and in the process 10 fractions of 10 ml each were collected. Elution with hexane plus 50% $CHCl_3$ gave 15.0 mg of deltaline (**7**) in fractions 6 and 7. Elution with hexane plus 50 to 70% $CHCl_3$ gave 14.5 mg of 14-acetyldictyocarpine (**8**) in fractions 8-10. The above experiment was finished in 1 h using a previously used vlc column (washed with $CHCl_3$ plus 10% MeOH). The total amount of solvents used was 100 ml. The identity of the isolated compounds was confirmed by their mp, mmp, tlc, and co-tlc behavior, and ^{1}H-nmr spectra. Deltaline (**7**): mp 185-187° (recrystallized from Et_2O), [lit. (19) mp 182-184°, 186.5-188°]. 14-Acetyldictyocarpine (**8**): mp 65-68° (amorphous), [lit. (19) mp 64-69.5°, amorphous].

SEPARATION OF HETISINE (**9**) AND HETISINONE (**10**).—A mixture of hetisine (16 mg) and hetisinone (16 mg) was applied to a vlc column (15 ml size) containing 5 g of Al_2O_3. Elution was carried out with a toluene/MeOH mixture, and in the process 20 fractions of 10 ml each were collected. Elution with toluene plus 3 to 5% MeOH afforded 16 mg of hetisinone (**10**) in fractions 7-11. Elution with toluene plus 6 to 8% MeOH afforded 15.5 mg of hetisine (**9**) in fractions 13 to 17. The above experiment was finished in 2 h using only 200 ml of solvents.

The alkaloids isolated were recrystallized and identified by comparison of their tlc, co-tlc, mp and mmp behavior, and ir spectra with those of authentic samples. Hetisine (**9**): mp 254-258° (recrystallized from Me_2CO/MeOH), [lit. (21) mp 259°]. Hetisinone (**10**): mp 267-269° (recrystallized from Me_2CO), [lit. (21) mp 267-269°].

SEPARATION OF DELPHININE (**11**) AND PYRODELPHININE (**12**) FROM A REACTION PRODUCT.—Pyrolysis of delphinine (**11**) (1.25 g) at 230-235° at 0.1 mm Hg gave 1.156 g of a mixture of pyrodelphinine, formed by elimination of the 8-acetoxy group as HOAc, and starting material. Passage through a short column of 20 g of Al_2O_3 (activity 3) gave 1.062 g of mixture. Analytical tlc examination of this mixture in various solvents on Al_2O_3 did not show much Rf difference; on silica gel with toluene plus 11%

Me_2CO a better separation was obtained (Rf difference 0.23). The reaction products were isolated by vlc (60 ml size) over 30 g of silica gel. Elution with toluene-Me_2CO (24:1) gave 896.0 mg of pyrodelphinine in fraction 5 and with toluene-Me_2CO (19:1) gave 108 mg of delphinine in fraction 7. This separation was efficient and accomplished in 3 h. The identification of the alkaloids isolated was achieved by comparison of their mp, mmp, tlc and co-tlc behavior, and ir spectra with those of authentic samples. Delphinine (**11**): mp 193-195° (recrystallized from EtOH), [lit. (19) mp 191-192°]. Pyrodelphinine (**12**): mp 208-211° (recrystallized from MeOH), [lit. (19) mp 208-212°].

ISOLATION OF 18-METHOXYGADESINE (**13**) FROM THE PRODUCTS OF REACTION OF DELCOSINE (**4**) WITH OsO_4.—To a solution of delcosine (64 mg) in pyridine (3 ml), a solution of OsO_4 (36 mg) in dioxane (3 ml) was added, and the mixture was stirred at room temperature for 8 h. After the usual work up (22), a mixture (67 mg) of three compounds with Rf values of 0.42, 0.19 (18-methoxygadesine), and 0.12 (delcosine) (Al_2O_3, $CHCl_3$ plus 0.5 % MeOH) resulted. The reaction products were isolated by vlc (15 ml size) over 12 g of Al_2O_3. Elution with petroleum ether-$CHCl_3$ (1:4) and $CHCl_3$ gave 34.0 mg of 18-methoxygadesine in fractions 4-6, which was crystallized from C_6H_6, mp 181-183°. Further purification by chromatography and recrystallization from C_6H_6 gave 18 mg, mp 184-185°: [lit. (13) mp 180-184°], $[\alpha]^{23}D$ +72.3° (*c*, 0.52, MeOH). The structure of the crystalline product was confirmed as 18-methoxygadesine (**13**) using ^{1}H-nmr, ^{13}C-nmr, and ir spectra. ^{1}H nmr ($CDCl_3$): δ 1.10 (3H, *t*, N-CH_2-CH_3), 3.30, 3.39, 3.40 (each 3H, *s*, OCH_3), 3.70 [1H, *m*, C(1) β-*H*], 4.13 [1H, *bs*, C(14)-β-*H*], 3.88, 3.95 [each 1H, *s*, C(6)-α-*H* and C(19)-H]. Because of an insufficient amount of other products, their characterization was not completed.

ISOLATION OF ACOFORESTININE (**14**) FROM THE PRODUCTS OF REACTION OF YUNACONITINE (**15**) WITH EtOH.—Yunaconitine (81 mg) was heated in absolute EtOH in a sealed tube at 130-135° for 24 h. The crude product (89 mg) obtained showed seven spots on the tlc (Al_2O_3, hexane-Me_2CO, 65:35) with Rf values 0.87, 0.73, 0.66, 0.52, 0.42, 0.38 (acoforestinine), and 0.33 (yunaconitine). The reaction products were isolated by vlc (15 ml size) over 10 g of Al_2O_3. Elution with hexane plus 14 to 15% Me_2CO afforded 67 mg of pure acoforestinine (**14**). The identity of acoforestinine (**14**) was confirmed by comparison of its tlc and co-tlc behavior, ir, ^{1}H-, and ^{13}C-nmr spectra with those of an authentic sample. ^{1}H nmr ($CDCl_3$): δ 0.59 (3H, *t*, J=7.5 Hz, OCH_2CH_3), 1.08 (3H, *t*, J=7.2 Hz, N-CH_2-CH_3), 3.25, 3.27, 3.31, 3.56 (each 3H, *s*, OCH_3), 3.90 (3H, *s*, Ar-OCH_3), 4.05 (1H, *d*, J=6 Hz), 6.91 (2H, *d*, J=9 Hz, aromatic protons), 8.01 (2H, *d*, J=9 Hz, aromatic protons). Because of an insufficient amount of companion products, their characterization was not completed.

SEPARATION OF 8-*O*-PROPYL-14-ANISOYLYUNACONINE (**16**) AND YUNACONITINE (**15**) FROM A REACTION PRODUCT.—Yunaconitine (30 mg) was heated in dry *n*-PrOH in a sealed tube at 135° for 24 h. The crude product (33 mg) showed four spots on tlc (Al_2O_3, hexane-Me_2CO, 65:35) with Rf values 0.45, 0.38 (8-*O*-propyl-14-anisoylyunaconine), 0.32 (yunaconitine), and 0.23. The reaction products were isolated by vlc (15 ml size) over 7 g of Al_2O_3. Elution with hexane plus 13% Me_2CO afforded 13 mg of pure 8-*O*-propyl-14-anisoylyunaconine (**16**). Elution with hexane plus 14% Me_2CO afforded 11 mg consisting mainly of yunaconitine in fractions 7 and 8. The yunaconitine from fractions 7 and 8 was purified by passing through a small vlc (15 ml size, 5 g of Al_2O_3) column. The structure of the 8-*O*-propyl-14-anisoylyunaconine was confirmed by its ^{1}H- and ^{13}C-nmr spectra. ^{1}H nmr ($CDCl_3$): δ 0.53 (3H, *t*, J=7 Hz, $OCH_2CH_2CH_3$), 1.11 (5H, *m*, $OCH_2CH_2CH_3$ and N-CH_2CH_3), 3.24, 3.27, 3.31, 3.52 (each 3H, *s*, OCH_3), 3.84 (3H, *s*, Ar-OCH_3), 4.04 [1H, *d*, J=6 Hz, C(6)-β-*H*], 4.80 [1H, *d*, J=5 Hz, C(14)-β-*H*], 6.90, 8.01 (4H, AB*q*, J=9 Hz, aromatic-*H*). The identity of the yunaconitine (**15**) was confirmed by comparison of its mp, mmp, tlc and co-tlc behavior, and ^{1}H-nmr spectrum with that of an authentic sample. Yunaconitine (**15**): mp 140-142° (recrystallized from EtOAc/hexane), [lit. (19) mp 141-143°].

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LITERATURE CITED

1. H.K. Desai, B.S. Joshi, A.M. Panu, and S.W. Pelletier, *J. Chromatogr.*, **322**, 723 (1985).
2. J.C. Coll, S.J. Mitchell, and G.J. Stokie, *Aust. J. Chem.*, **30**, 1859 (1977).
3. B.F. Bowden, J.C. Coll, S.J. Mitchell, and G.J. Stokie, *Aust. J. Chem.*, **31**, 1303 (1978).
4. N.M. Targett, J.P. Kilocyne, and B. Green, *J. Org. Chem.*, **44**, 4962 (1979).
5. S.W. Pelletier, B.S. Joshi, and H.K. Desai, in: "Techniques for Isolation of Alkaloids," "Advances in Medicinal Plant Research," Ed. by A.J. Vlietinck and R.A. Dommisse, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1985, pp. 153-196.
6. H.K. Desai and S.W. Pelletier, unpublished work.

7. S.D. Sastry and G.R. Waller, *Chem. Ind., (London),* 429 (1978).
8. S.W. Pelletier, R.S. Sawhney, H.K. Desai, and N.V. Mody, *J. Nat. Prod.*, **43**, 395 (1980).
9. B.S. Joshi, J.A. Glinski, H.P. Chokshi, S.Y. Chen, S.K. Srivastava, and S.W. Pelletier, *Heterocycles,* **22**, 2037 (1984).
10. S.W. Pelletier, O.D. Dailey, Jr., N.V. Mody, and J.D. Olsen, *J. Org. Chem.*, **46**, 3284 (1981).
11. S.W. Pelletier, N.V. Mody, and H.K. Desai, *J. Org. Chem.*, **46**, 1845 (1981).
12. S.W. Pelletier, J. Finer-Moore, R.C. Desai, N.V. Mody, and H.K. Desai, *J. Org. Chem.*, **47**, 5290 (1982).
13. A.G. Gonzalez, G. de la Fuente, and O. Munguia, *Heterocycles,* **20**, 409 (1983).
14. V. Boido, O.E. Edwards, K.L. Handa, R.J. Kolt, and K.K. Purushothaman, *Can. J. Chem.*, **62**, 778 (1984).
15. S.W. Pelletier, S.K. Srivastava, B.S. Joshi, and J.D. Olsen, *Heterocycles,* **23**, 331 (1985).
16. C.A. Grob, H.R. Kiefer, H. Lutz, and H. Wilkens, *Tetrahedron Letters,* 2901 (1964).
17. W.C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923, (1978).
18. B. Loev and M. Goodman, *Chem. Ind. (London),* 2026 (1967).
19. S.W. Pelletier, N.V. Mody, B.S. Joshi, and L.C. Schramm, in: "Alkaloids: Chemical and Biological Perspectives." Ed. by, S.W. Pelletier, vol. 2, chap. 5, John Wiley & Sons, New York, 1984, pp. 205-462.
20. H. Hikino, Y. Kuroiwa, and C. Konno, *J. Nat. Prod.*, **46**, 178 (1983).
21. S.W. Pelletier, J.A. Glinski, B.S. Joshi, and S.Y. Chen, *Heterocycles,* **20**, 1347 (1983).
22. S.W. Pelletier, H.K. Desai, J. Finer-Moore, and N.V. Mody, *Tetrahedron Lett.*, **23**, 4229 (1982).

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