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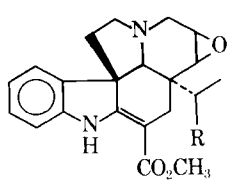
Catharanthus Alkaloids XXXII: Isolation of Alkaloids from *Catharanthus trichophyllus* Roots and Structure Elucidation of Cathaphylline

G. A. CORDELL and N. R. FARNSWORTH x

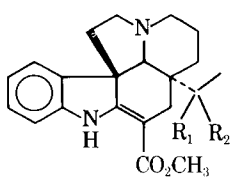
Abstract □ Further examination of the cytotoxic alkaloid fractions of *Catharanthus trichophyllus* roots afforded nine alkaloids. Two of these alkaloids, lochnericine and horhammericine, are responsible for part of the cytotoxic activity. The structure elucidation of cathaphylline, a new β-anilino acrylate derivative, is described.

Keyphrases □ *Catharanthus* alkaloids—isolation of nine alkaloids from *Catharanthus trichophyllus* roots, cytotoxic activity screened, structure elucidation of cathaphylline □ Alkaloids—isolated from *Catharanthus trichophyllus*, screened for cytotoxic activity □ Cathaphylline—isolated from *Catharanthus trichophyllus*, structure elucidated, cytotoxic activity screened □ Cytotoxicity—nine alkaloids isolated from *Catharanthus trichophyllus* screened

The cytotoxic and antitumor activities of the alkaloid fraction of *Catharanthus trichophyllus* were described previously (1, 2). However, the alkaloids isolated from this plant in those studies were devoid of antitumor and/or cytotoxic activity. The present phytochemical study dealt with the isolation of the two known alkaloids, lochnericine (I) and horhammericine (II), which were found to be cytotoxic. In addition, the isolation of several other bases from *C. trichophyllus* is reported, including a new indole alkaloid, cathaphylline, for which Structure X is proposed.



I: R = H
II: R = OH



III: R_1R_2 = O
IV: R_1 = H, R_2 = $OCOCH_3$
V: R_1 = H, R_2 = OH
IX: R_1 = H, R_2 = H

EXPERIMENTAL[1]

Plant Material—The coarsely milled, air-dried roots[2] of *C. trichophyllus* (Bak.) Pich. (Apocynaceae) used were collected in Madagascar during 1969.

Preparation of pH Gradient Fractions—Fractionation of the crude alkaloids from this plant and the pH gradient separation of the Extract N alkaloids were described previously (2).

Chromatographic Separation of N-3.0 Alkaloids—Fraction

[1] Melting points were determined using an American Optical Instrument Co. model 569 instrument and are uncorrected. Specific rotations were measured using a Carl Zeiss optical polarimeter (Merz Optical Instruments, Chicago, Ill.). UV spectra were recorded in ethanol using a Beckman model DB-G grating spectrophotometer. IR spectra were measured as potassium bromide pellets *versus* air with a Beckman model IR-18A spectrophotometer. Low-resolution mass spectra were recorded at 70 ev using a Hitachi Perkin-Elmer model RMU-6D mass spectrometer. High-resolution mass spectra were recorded using a double-focusing mass spectrometer, model 110 (Consolidated Electro Dynamics Co.), operating at 70 ev. NMR spectra were determined in deuterochloroform, with tetramethylsilane as the internal standard, using a Varian model T-60A spectrometer. Column chromatography was carried out using silica gel PF_{254} (E. Merck, Darmstadt, West Germany). Routine analytical TLC was carried out using silica gel PF_{254} on 0.25-mm layers (E. Merck), and preparative TLC was performed using silica gel PF_{254} on 2-mm layers. Resolved components were detected by quenching under 254-nm UV light and visualized with ceric ammonium sulfate reagent. Appropriate zones were removed from the plates and the components were purified by filtered elution with methanol and evaporation of the solvent *in vacuo*. The residue was taken up in chloroform or ethyl acetate and filtered, and the filtrate was evaporated *in vacuo*. Solvent systems used in this study were: A, 1-butanol–acetic acid–water (4:4:1); B, 1-butanol–acetic acid–water (10:1:1); C, benzene–triethylamine (9:1); D, ethyl acetate–absolute ethanol (3:1); and E, 1-butanol–acetic acid–water (10:10:1).

[2] Obtained from the Curran Corp., South Hackensack, N.J. Voucher specimens representing the collection are deposited in the Herbarium of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, Ill. The identification was confirmed by N. R. Farnsworth.

N-3.0 (8.7 g) from the pH gradient fractionation of Extract N was intimately mixed with silica–diatomaceous earth[3] (3:1)(8.7 g) and placed at the top of a chromatographic column slurry packed with 500 g of silica and petroleum ether. Elution was carried out with petroleum ether; 2100 fractions (10 ml each) were collected, analyzed by TLC, and combined on the basis of similarities or differences in the separation patterns.

Isolation and Identification of (−)-Minovincine (III), (−)-Echitovenine (IV), and Lochnericine (I)—Fractions 818–1125 from the chromatographic separation of N-3.0 were combined and taken to dryness *in vacuo*. The combined total fraction (2.5 g) was chromatographed over a silica (175 g) column slurry packed with a chloroform–benzene (1:1) mixture. Elution with the same eluent afforded 580 fractions (10 ml each), which were combined on the basis of TLC patterns.

Fractions 11–20 from the column were combined, taken to dryness *in vacuo* (0.065 g), and further separated by preparative TLC, eluting with 1-butanol–acetic acid–water (10:1:1). A band at R_f 0.75 was removed, purified, and subjected to preparative TLC, eluting with benzene–triethylamine (9:1). The band at R_f 0.47 was removed and purified to afford (−)-lochnericine (I), which was recrystallized from ethanol to afford 38.2 mg of white needles, mp 190–192°, $[\alpha]_D^{23}$ −510° (c 0.1, chloroform).

The alkaloid was homogeneous by TLC using Solvent Systems A–D. It exhibited R_f values of 0.52, 0.75, 0.48, and 0.68, respectively, and a strong dark-blue chromogenic response with the ceric ammonium sulfate reagent. The UV spectrum showed λ_{max} (ethanol) at 299 (log ϵ 4.12) and 328 (4.30) nm. A mass spectrum of the alkaloid (3, 4) gave ions at *m/e* 352 (M^+, 90%), 323 (17), 214 (45), 138 (100), and 108 (60). An IR spectrum (potassium bromide) exhibited principal absorptions at ν_{max} 3380, 1675, and 1615 cm^{-1}. NMR data were identical with those obtained for lochnericine (3).

Fractions 21–30 from the column were combined and taken to dryness *in vacuo* (0.241 g), and the fraction was subjected to preparative TLC, eluting with 1-butanol–acetic acid–water (10:1:1). Four bands were removed. One, at R_f 0.75, was further purified and shown to be lochnericine (39 mg), identical with the previously isolated material. A second band at R_f 0.25 was purified to afford (−)-minovincine (III) as an amorphous powder (0.49 g), $[\alpha]_D^{23}$ −480° (c 0.1, chloroform).

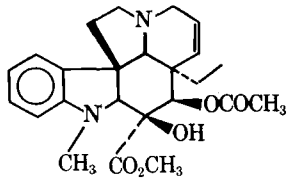
This alkaloid was homogeneous by TLC using Solvent Systems B, C, and D; it exhibited R_f values of 0.22, 0.51, and 0.66, respectively, as well as an initial strong dark-blue chromogenic response with ceric ammonium sulfate reagent. A UV spectrum showed λ_{max} (ethanol) at 298 (log ϵ 4.09) and 328 (4.28) nm. The mass spectrum exhibited ions at *m/e* 352 (M^+, 41%), 309 (23), 214 (26), and 138 (100). An IR spectrum (potassium bromide) showed principal absorptions at ν_{max} 3390, 1715, 1672, and 1610 cm^{-1}. The NMR spectrum revealed two strong singlets: for the C-18 methyl group at δ 1.87 ppm and for the carbomethoxyl group at δ 3.76 ppm. These data correspond to those previously reported for minovincine (4).

A third band at R_f 0.6 was separated by preparative TLC, eluting with chloroform. A band at R_f 0.55 was removed and purified to afford (−)-echitovenine (IV), which was crystallized from aqueous ethanol to give white needles (13 mg), mp 170–172°, $[\alpha]_D^{23}$ −436° (c 0.1, chloroform). This isolate was homogeneous by TLC using Solvent Systems B, C, and D; it exhibited R_f values of 0.53, 0.50, and 0.68, respectively, and an initial dark-blue chromogenic response with the ceric ammonium sulfate reagent.

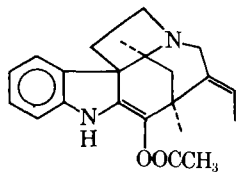
A UV spectrum of the isolate exhibited λ_{max} (ethanol) at 298 (log ϵ 4.09) and 328 (4.28) nm.

The mass spectrum of the isolate displayed ions at *m/e* 396 (M^+, 18%), 337 (12), 309 (8), and 182 (100). The IR spectrum showed principal absorptions at ν_{max} 3390, 1715, 1680, 1615, and 1245 cm^{-1}. An NMR spectrum showed a doublet, J = 7 Hz, for the C-18 protons at δ 1.00 ppm and a quartet, J = 7 Hz, for the C-19 proton at δ 4.76 ppm. The protons of the carbomethoxyl and acetyl groups were observed at δ 3.80 and 2.16 ppm, respectively. The compound exhibited identical spectral data with an authentic sample of (+)-echitovenine (5). This study is the first reported isolation of the (−)-isomer of echitovenine.

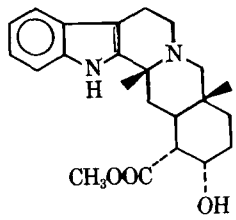
Chromatographic Separation of N-3.5 Alkaloids—Fraction N-3.5 (5.55 g) from the pH gradient fractionation of Extract N was chromatographed over a column containing silica (400 g) packed in ethyl acetate–95% ethanol (3:1). A total of 266 fractions (10 ml each) was collected from the column and combined on the basis of their TLC patterns.



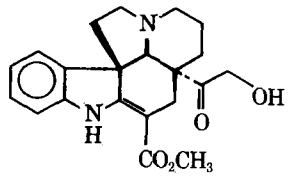
VI



VII



VIII



X

Isolation of Minovincinine (V) and Horhammericine (II)—Fractions 1–30 from the chromatographic separation of N-3.5 were combined, and the total fraction (1.5 g) was chromatographed over a column of silica (150 g) packed in chloroform–benzene (1:1). Elution with the same solvent mixture afforded 480 fractions (10 ml each), which were combined on the basis of their TLC patterns. Combined fractions 268–282 (0.128 g) were subjected to preparative TLC, eluting with 1-butanol–acetic acid–water (10:1:1). Two bands at R_f 0.55 and 0.35 were removed and purified. Material from the less polar band was crystallized from benzene to afford white needles (58 mg) of horhammericine (II), mp 138–140°, $[\alpha]_D^{23}$ −395° (c 0.1, chloroform).

The compound was homogeneous by TLC using Solvent Systems B, C, and D; it exhibited R_f values of 0.55, 0.35, and 0.58, respectively, and a strong dark-blue chromogenic response with the ceric ammonium sulfate reagent. A UV spectrum showed λ_{max} (ethanol) at 299 (log ϵ 4.14) and 328 (4.32) nm. The mass spectrum displayed ions at *m/e* 368 (M, 40%), 350 (53), 323 (12), 214 (54), and 154 (100). An IR spectrum (potassium bromide) showed principal absorptions at ν_{max} 3350, 1675, and 1610 cm^{-1}. The NMR spectrum of the isolate showed a doublet, J = 7 Hz, for the C-18 protons at δ 1.10 ppm as well as a quartet, J = 7 Hz, for the C-19 proton at δ 3.80 ppm. A mixed melting-point determination with authentic horhammericine showed no depression. Acetylation (acetic anhydride in pyridine at room temperature for 12 hr) afforded acetylhorhammericine as fine needles, mp 176–178°, whose mass spectrum exhibited ions at *m/e* 410 (M^+, 82%), 351 (100), 350 (80), 214 (80), and 196 (90).

Attempts to crystallize the material from the more polar band were unsuccessful. The white, amorphous minovincinine (V) (0.35 g), $[\alpha]_D^{23}$ −412° (c 0.1, chloroform), was homogeneous by TLC in Solvent Systems B, C, and D; it exhibited R_f values of 0.35, 0.38, and 0.60, respectively, and a dark-blue response with the ceric ammonium sulfate reagent. The UV spectrum showed λ_{max} (ethanol) at 299 (log ϵ 4.07) and 329 (4.26) nm.

A mass spectrum of the isolate exhibited ions at *m/e* 352 (M^+, 18%), 309 (2.6), and 140 (100). An IR spectrum (potassium bromide) displayed principal absorptions at ν_{max} 3460, 3380, 1680, and 1612 cm^{-1}. The NMR spectrum showed a doublet, J = 7 Hz, for the C-18 protons at δ 0.94 ppm and a quartet, J = 7 Hz, for the C-19 proton at δ 3.45 ppm. Acetylation (acetic anhydride in pyridine at room temperature for 12 hr) afforded (−)-echitovenine

[3] Celite.

Table I—Mass Spectral Comparison of β-Anilino Acrylate Derivatives

Compound	Parent Ion	Ion A	Ion B	Ion C	Ion D
			m/e		
IX	338	214	124	29	309
I	352	214	138	29	323
II	368	214	154	45	323
III	352	214	138	43	309
IV	396	—	182	87	309
V	354	—	140	45	309
Cathaphylline	368	214	154	59	309

(IV), which exhibited identical melting point, mixed melting-point, TLC, mass spectral, UV, IR, and NMR properties as the material isolated from Fraction N-3.0.

Isolation and Identification of Vindorosine (VI)—Fractions 31–51 from the chromatographic separation of N-3.5 were combined and taken to dryness *in vacuo*, and the total fraction (3.2 g) was chromatographed over a column of silica (350 g) packed in chloroform–benzene (3:1). Elution of the column with the same solvent mixture afforded 500 fractions (10 ml each), which were combined on the basis of TLC patterns. Combined fractions 241–310 (0.330 g) were subjected to preparative TLC on silica gel plates (2 mm), eluting with 1-butanol–acetic acid–water (10:1:1).

One band at R_f 0.25 was removed, purified, and rechromatographed on silica, eluting twice with benzene–triethylamine (19:1). Two bands at R_f 0.35 and 0.25 were removed and purified. The less polar band was crystallized from benzene–petroleum ether to afford vindorosine (VI) (0.181 g), mp 165–166°. This isolate was essentially homogeneous by TLC, displaying a highly characteristic orange-yellow chromogenic response with the ceric ammonium sulfate reagent and giving R_f values of 0.40, 0.25, 0.38, and 0.22 in Solvent Systems A, B, C, and E, respectively. Trace contamination with vindoline was observed (6). The IR, UV, NMR, and mass spectral data were identical with those of an authentic sample of vindorosine. A mixed melting-point determination showed no depression.

The more polar band was rechromatographed on silica, eluting three times with benzene–triethylamine (98:2). One major band at R_f 0.28 was removed and purified. Attempts to crystallize this material (23.6 mg) were unsuccessful. It exhibited a high negative rotation, $[\alpha]_D^{23}$ −438° (c 0.1, chloroform). The compound was homogeneous by TLC, using Solvent Systems A, B, C, and E; it exhibited R_f values of 0.35, 0.25, 0.44, and 0.32, respectively, and an initial strong dark-blue response with the ceric ammonium sulfate reagent.

The UV spectrum of the isolate showed λ_{max} (ethanol) 299 (log ε 4.14) and 328 (4.31) nm. The mass spectrum showed ions at *m/e* 368 (M^+, 65%), 337 (82), 309 (31), 214 (45), and 154 (100). An IR spectrum showed principal absorptions at ν_{max} 3450, 3350, 1710, 1675, and 1615 cm^{-1}. The NMR spectrum showed a complex multiplet in the δ 6.70–7.40-ppm region corresponding to four aromatic protons. The carbomethoxyl group gave rise to a singlet at δ 3.75 ppm, and the *N* proton was observed at δ 8.81 ppm; a two-proton broad doublet was found at δ 4.13 ppm. The structure elucidation of this new alkaloid, which has been given the trivial name cathaphylline, will be described (*vide infra*).

Isolation of Akuammicine (VII)—Fractions 150–260 from the chromatography of N-3.5 were combined, and a portion of this fraction (0.020 g) was subjected to preparative TLC, eluting with benzene–triethylamine (9:1). One band at R_f 0.36 was removed, purified, and chromatographed on silica gel, eluting with ethyl acetate–methanol (3:1). The band at R_f 0.20 was removed, purified, and chromatographed on silica gel, eluting with 1-butanol–acetic acid–water (8:1:1). A second major band at R_f 0.26 was removed and purified to a white amorphous powder (1.2 mg). This product was homogeneous by TLC, having R_f values of 0.62, 0.18, and 0.25 using Solvent Systems A, B, and D, respectively. The mass spectrum gave *m/e* 322 (M^+, 100), 263 (77), and 121 (33). The mass spectral and TLC data were identical with those of authentic akuammicine (VII).

Table II—High-Resolution Mass Spectral Analysis of Cathaphylline and Acetylcathaphylline

Cathaphylline			Acetylcathaphylline		
m/e	Percent	Molecular Formula	*m/e*	Percent	Molecular Formula
368	65	$C_{21}H_{24}N_2O_4$	410	54	$C_{23}H_{26}N_2O_5$
337	82	$C_{20}H_{21}N_2O_3$	337	15	$C_{20}H_{21}N_2O_3$
309	31	$C_{19}H_{21}N_2O_2$	309	36	$C_{19}H_{21}N_2O_2$
214	45	$C_{13}H_{12}NO_2$	214	41	$C_{13}H_{12}NO_2$
154	100	$C_8H_{12}NO_2$	196	100	$C_{10}H_{14}NO_3$

Chromatographic Separation of N-6.5 and N-7.0 Alkaloids—Fractions N-6.5 and N-7.0 were combined, and the total fraction (2.92 g) was chromatographed over a column of silica (220 g) packed in ethyl acetate–95% ethanol (3:1). A total of 290 fractions (10 ml each) was collected and combined on the basis of TLC patterns.

Isolation of Pseudoyohimbine (VIII)—Fractions 70–88 from the chromatography of N-6.5 and N-7.0 were combined, and the total fraction (0.16 g) was chromatographed on silica, eluting with 1-butanol–acetic acid–water (10:1:1). Four bands were removed from the plate and purified. A compound obtained from the band at R_f 0.42, *m/e* 296 (M^+), was new but thus far has resisted structure elucidation.

A second purified band crystallized on standing to afford pseudoyohimbine (VIII) (0.014 g), mp 266–267°. The compound was homogeneous by TLC, using Solvent Systems B, C, and D; it exhibited R_f values of 0.25, 0.05, and 0.15, respectively, and a yellow chromogenic response with the ceric ammonium sulfate reagent. The UV, IR, NMR, mass spectral, and specific rotation data were identical with those of authentic pseudoyohimbine. A mixed melting-point determination showed no depression.

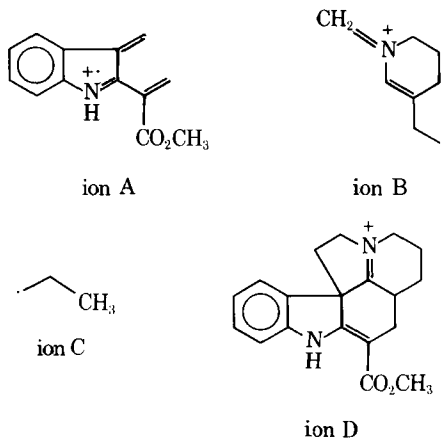
Cytotoxic Activities—Echitovenine, (−)-minovincine, cathaphylline, lochnericine, and horhammericine were examined for their activity against Eagle's 9KB carcinoma of the nasopharynx in cell culture using established protocols (7). Lochnericine and horhammericine were found to be active (ED_{50} 1.1 μg/ml).

DISCUSSION

The retro Diels–Alder fragmentation pathway, which is characteristic for the mass spectra of many aspidosperma-type alkaloids, proved to be of considerable diagnostic value. For the purposes of discussing the elucidation of the structure of cathaphylline, four ions were considered important in in the fragmentation patterns of the β-anilino acrylate alkaloids isolated in this study. Ions A and B arise from the retro Diels–Alder fragmentation of ring C and fission of the 5,6-bond. Ions C and D arise by loss of the two-carbon side chain.

A comparison of the *m/e* values for these ions is shown in Table I for the compounds isolated [and vincadifformine (IX) for comparison]. Substitution in the indole portion leads to a change in the mass of A, whereas substitution in the piperidine ring leads to an increase in the mass of B and D. Substitution solely in the side chain affords an increase in mass for B and C from the "standard" values of 124 and 29 mass units (mu), respectively.

On this basis, examination of these four fragments in the case of



cathaphylline indicates: (*a*) no substitution in the indole ring, (*b*) no substitution in the piperidine ring, and (*c*) an addition of 30 mu in the two-carbon side chain.

The high-resolution mass spectral data for cathaphylline (Table II) indicate that four oxygen atoms are present: two in the carbomethoxyl group and two in the side chain. The side chain was shown to have a molecular formula of $C_2H_3O_2$ (M^+ – ion D).

Treatment of cathaphylline with acetic anhydride and pyridine afforded a monoacetate, showing three-proton singlets at δ 3.78 and 1.99 ppm in the NMR spectrum. The mass spectrum shows *m/e* 410 (M^+), in which ion B is shifted by 42 mu to *m/e* 196, indicating the presence of a hydroxyl group in the side chain. Confirmation of this point is found in the IR spectrum of cathaphylline, which shows a band at 3450 cm^{-1}. Two distinct possibilities exist for the functionality of the two-carbon side chain, a hydroxyaldehyde or a hydroxyketone. In the NMR spectrum of cathaphylline, no signal was observed which could be assigned to an aldehyde function. In the mass spectra of cathaphylline and acetylcathaphylline, a fragment ion was observed at *m/e* 337, corresponding to initial losses of CH_3O and $C_3H_5O_2$, respectively. Therefore, the acetylated functional group is at the terminal carbon of the side chain, *i.e.*, C-18.

In support of this assignment of a hydroxyketone to the side chain, the ion at *m/e* 337 loses CO to give *m/e* 309 (ion D); in the NMR spectrum, a two-proton broadened doublet was observed at δ 4.13 ppm, corresponding with the C-18 protons of the hydroxymethyl group. This signal became two doublets, δ 4.84 and 4.34 ppm (J = 16.5 Hz), in the NMR spectrum of acetylcathaphylline. The high negative specific rotation is characteristic of α-methylene indole alkaloids having the absolute stereochemistry shown. Therefore, Structure X is suggested for cathaphylline.

Akuammicine, an alkaloid originally isolated from *Picralima nitida* (8, 9), has also been isolated from *Vinca erecta* (10), *V. minor* (11), and *C. roseus* (12, 13). Vindorosine (VI) is apparently characteristic of the genus *Catharanthus*, rather than *Vinca*, having been isolated from *C. pusillus* (14), *C. roseus* (6), and *C. trichophyllus* (1). Lochnericine has been obtained from *Amsonia angustifolia* (15), *A. tabernaemontana* (16), and *C. roseus* (17–19). (+)-Echitovenine was reported previously only from *Alstonia venenata* (5). Horhammericine was isolated previously from *C. lanceus* (20, 21). (−)-Minovincinine was isolated previously from *V. minor* (22) and *V. erecta* (4), and the (+)-isomer was obtained from *A. venenata* (5). Minovincine was previously obtained only from *V. erecta* (4). Pseudoyohimbine was not reported previously from *Catharanthus* or *Vinca* species.

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