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DEOXYHARRINGTONINE, A NEW ANTITUMOR ALKALOID FROM *CEPHALOTAXUS*: STRUCTURE AND SYNTHETIC STUDIES*

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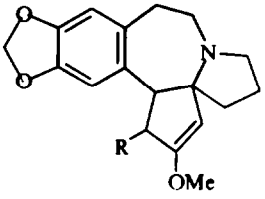
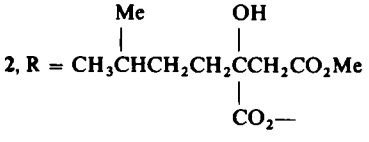
Abstract—A new alkaloid (proposed name, deoxyharringtonine) with significant antileukemic activity has been isolated from *Cephalotaxus harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata. Its structure has been established as either **2** or **3** by chemical and spectral methods. Total synthesis of the dicarboxylic acid side chain and esterification of its primary carboxyl group with natural cephalotaxine (**1**) yields **3**; therefore, deoxyharringtonine must be **2**.

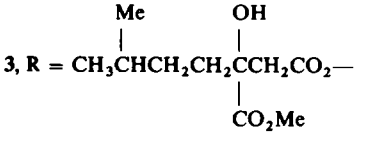
THREE ANTITUMOR ALKALOIDS—harringtonine, homoharringtonine, and isoharringtonine—have previously been isolated from *Cephalotaxus harringtonia* plant materials and characterized.[1] These alkaloids, which show significant inhibitory activity



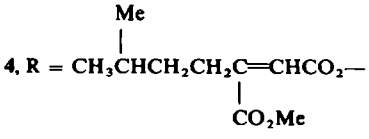
1, R = HO—



2, R = $CH_3CHCH_2CH_2CCH_2CO_2Me$ (Me; OH; CO_2—)



3, R = $CH_3CHCH_2CH_2CCH_2CO_2$— (Me; OH; CO_2Me)



4, R = $CH_3CHCH_2CH_2C{=}CHCO_2$— (Me; CO_2Me)

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against experimental lymphoid leukemia systems L1210 and P388 in mice,* are all esters of cephalotaxine (**1**). Cephalotaxine[1a, 1b, 2] accounts for approximately 50% of the total alkaloids present in *Cephalotaxus* plant extracts, but it is inactive. We now report the structure of a fourth cephalotaxine ester that we propose naming deoxyharringtonine (**2**) because of its relationship to harringtonine.[1c, 1d] This new alkaloid has antileukemic activity of the same order of magnitude as that previously reported for the other three cephalotaxine esters.[1a, 1c]

Since the alkaloid esters of *Cephalotaxus* occur naturally in such small quantities,[3] extensive biological testing might require additional sources of these compounds. Therefore, we decided to synthesize the dicarboxylic acid moieties and esterify them in the proper manner with natural cephalotaxine, which had already been isolated in substantial amounts. This program, if successful, would provide a temporary solution to the availability problem and permit continued testing. Accordingly, we describe the synthesis of the R-group of deoxyharringtonine (**2**), along with our attempts to esterify it with cephalotaxine.

A brief statement concerning the isolation of alkaloid **2** is given here as a detailed report will be published soon.[4] The IR spectrum of **2** suggested that it was an alkaloid ester with one free hydroxyl group rather than two as in the other three *Cephalotaxus* esters.[1c, 1d] High-resolution mass-spectral analysis gave a molecular weight of 515·246 (Calc. 515·251) which is 16 mass units (one oxygen) less than the molecular ion displayed by harringtonine and isoharringtonine and is 30 units (CH_2O) less than that of homoharringtonine. Major fragmentations of **2** give *m/e* 314 ($C_{18}H_{20}NO_4$), 298 ($C_{18}H_{20}NO_3$), 266 ($C_{17}H_{16}NO_2$) and 150 derived from the cephalotaxine (**1**) moiety. Significant ions in the spectrum of **2** at *m/e* 173, 159, 141, 99 and 81 are derived from the acyl moiety as revealed by spectra of compounds **9**, **10**, and **11** (Scheme 1).

The NMR spectrum of deoxyharringtonine is distinctive yet very similar to spectra of the other three alkaloid esters from this plant.[1c, 1d] Since the NMR spectrum in DMSO shows one exchangeable proton as a singlet at δ 4·73, there is only one free hydroxyl group in **2** and it is tertiary.[5]

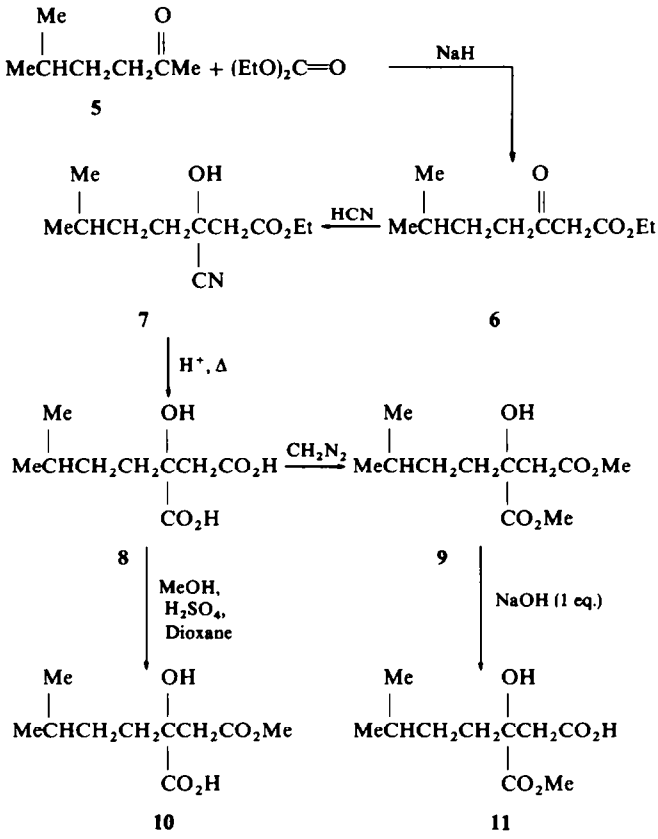
Base-catalyzed transesterification of **2** further pinpoints its structure; cephalotaxine (**1**) and a dimethyl ester with the same structure as synthetic compound **9**, but presumably optically active,† are the products of this reaction. Synthetic compound **9** and dimethyl ester **9** from basic transesterification of **2** give identical IR, NMR, and mass spectral data. Of course, synthetic **9** is racemic. Because of these data, the only elusive structural feature of **2** that remains, other than its absolute configuration, concerns which dicarboxylic acid carboxyl group (primary or tertiary) is esterified with the hydroxyl group of cephalotaxine.

The NMR spectrum of **9** reveals carbomethoxyl signals at δ 3·64 and 3·77, while half esters **10** and **11** give corresponding signals at δ 3·68 and 3·76, respectively. Logically, the downfield signal of these two is due to the tertiary carbomethoxyl groups. This evidence is supported by the spectrum of trimethyl citrate, in which

* Assays were performed under the auspices of Drug Research and Development, National Cancer Institute (formerly Cancer Chemotherapy National Service Center). The procedures were described in *Cancer Chemother. Rep.* **25**, 1 (1962).

† Not enough material of sufficient purity for a meaningful ORD analysis was available.

SCHEME 1



the tertiary carbomethoxyl signal appears at δ 3·78 and the primary carbomethoxyl signals appear at δ 3·64. It can also be seen that methylation of **10** shifts the existing primary carbomethoxyl signal from δ 3·68 to 3·64. In the spectrum of **2**, therefore, the δ 3·64 (downfield of the two signals) is likely due to the vinyl methoxyl while the δ 3·53 signal results from an upfield shift of the δ 3·68 primary carbomethoxyl signal of **10**; thus in **2**, cephalotaxine is shown to be esterified with the tertiary carboxyl group of the acid moiety. This important detail provided the basis for establishing that the other cephalotaxine esters, harringtonine, homoharringtonine and isoharringtonine,[1d] are also derived by esterification of cephalotaxine with the tertiary carboxyl group of appropriate dicarboxylic acids.

We undertook the synthesis of half esters **10** and **11** (previously unknown compounds) and the esterification of each of them with cephalotaxine isolated from the plant extract. This partial synthesis, in addition to confirming the structure of deoxyharringtonine, would enhance our supply of **2** for biological testing. The synthesis of dicarboxylic acid **8** (Scheme 1) is straightforward, and yields are good when NaH is used in the initial condensation reaction.[6] The absence of a nitrile absorption band in the IR spectrum of **7** was expected because the nitrile group is attached to an oxygen-bearing carbon.[7]

Half esters **10** and **11** are prepared by reactions that attack the least hindered

carboxyl group. Mass spectral, NMR and IR data support the structures shown for compounds **9**, **10** and **11**. An interesting fragmentation pattern is observed in the mass spectra of **10** and **11**. Ions involved are those of *m/e* 173 (M − 45) and *m/e* 159 (M − 59), corresponding to loss of —CO_2H and —CO_2Me, respectively. Relative intensities of ion 173 to 159 (3:1 in **10** and 1:8 in **11**) clearly demonstrate that preferred cleavage occurs at the tertiary position. The spectrum of **2** displays *m/e* 173 and 159 ions in essentially the same ratio (3:1) as that of **10**; these data support NMR results and the conclusion that deoxyharringtonine consists of cephalotaxine esterified with the tertiary carboxyl of **10** as depicted in structure **2**. An *m/e* 159 ion in the spectrum of **10** could arise by splitting off the carbomethoxyl, but this reasoning does not account for a 159 ion in the spectrum of **2**. We feel that this ion results from ester scrambling under electron impact in both spectra. A certain amount of cleavage removing the cephalotaxine fragment of **2** must have occurred before scrambling of the methyl ester takes place. Simple cleavage could produce the *m/e* 173 ion from **11**, but it might also arise from ester scrambling followed by cleavage as well. The base peak *m/e* 99 in the spectra of **8**, **9**, **10** and **11** is probably $(CH_3)_2CHCH_2CH_2\overset{+}{C}{=}O$; the base peak *m/e* 298 in the spectra of **2**, **3** and **4** is the stabilized allylic cation from the cephalotaxine-minus-hydroxyl (315–17) moiety.

Synthesis of alkaloid ester **3** from the acid chloride of **11** and cephalotaxine proceeded readily as expected. Even though two diasteromers are formed because **11** is racemic, spectral data reveal that neither of them is deoxyharringtonine (**2**). These two diastereomers give rise to the duplicate signals of slightly differing chemical shifts and multiplicities observed for the vinyl, methine and aromatic protons in the NMR spectrum of **3**. These data thereby provide important evidence that structure **2** is correct for deoxyharringtonine because it is the only alternative.

A major byproduct of the reaction producing **3** is the corresponding dehydrated ester **4**. We have no evidence to distinguish whether dehydration occurs during acyl chloride preparation or during the actual esterification reaction; either route seems possible. Absence of a chiral center in the acyl moiety of **4** precludes formation of a diastereomeric mixture* and this point is confirmed by the NMR spectrum. Although alkaloid esters **3** and **4** have been subjected to preliminary antitumor screening, available results are inadequate for a meaningful interpretation.

Attempts to synthesize deoxyharringtonine by esterifying cephalotaxine with the hindered tertiary carboxyl group of **10** *via* the acid chloride have given complex mixtures which contain none of the desired product. We are actively pursuing other approaches to the problem and hope to report a solution soon.

EXPERIMENTAL

A Beckman DK-2A† recording spectrophotometer was used to record UV spectra, and IR analyses were done on 1% solutions in $CHCl_3$ with a Perkin-Elmer Model 137 instrument. NMR spectra were obtained on $CDCl_3$ solutions (unless otherwise specified) with a Varian HA-100 spectrometer, and chemical

* Only the positional isomer indicated in **4** was detected here. But when **9** is subjected to purposeful dehydration with $SOCl_2$ and pyridine, both possible positional isomers (double bond conjugated with both carboxyl groups as in **4**, and double bond conjugated with only the tertiary carboxyl group) are isolated in a 55:45 ratio.

† The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

shifts are given relative to internal TMS. Mass spectral analyses were performed at 70 eV with a Nuclide 12–90 G or with a CEC 21–492–1 spectrometer, and ORD spectra were recorded with a Carey Model 60 spectropolarimeter. M.ps were determined with a Fisher–Johns block and are uncorrected.

All compounds were analyzed by TLC with appropriate solvent systems on either 0·25 mm Silica Gel G plates or on Brinkmann precoated 0·25 mm Silica Gel F-254 plates. Spots were visualized either by staining them with iodine vapour or by spraying the plates with an ethanolic solution of bromthymol blue. All preparative separations were made on 1 mm Silica Gel G layers with bromthymol blue visualization. Ether and $CHCl_3$ extracts were routinely dried over $MgSO_4$.

Deoxyharringtonine (**2**). The original material extracted from the plant with 95% EtOH was dissolved in 5% tartaric acid aq; extraction with $CHCl_3$ removed the neutral and acidic components. Basification of the resultant aqueous phase, followed by a second $CHCl_3$ extraction, afforded a crude alkaloid mixture that was subjected to a 10-tube countercurrent distribution between $CHCl_3$ and pH 5 McIlvaine buffer.[8] Deoxyharringtonine (**2**), along with some other alkaloids, remained in tubes 1 and 2. Finally, 2 was purified by column chromatography and subsequent prep TLC of material from selected column fractions; generally, a yield of <0·01% of the plant dry weight or about 2% of the crude alkaloid mixture was realized. Although apparently pure as evidenced by a single TLC spot and clean NMR spectrum, deoxyharringtonine existed as an amorphous solid, which could not be induced to crystallize: IR 3600 (—OH), 1740 (ester C=O), 1650 (trisubstituted double bond) and 925–950 cm^{-1} (methylenedioxy); λ_{max}^{EtOH} 290 μm (log ε = 3·66) and 261 μm (log ε = 2·86); $[\alpha]_D^{26°}$ = − 151° (*c* 0·03 EtOH) and $[\alpha]_D^{26°}$ = − 119° (*c* 0·6 $CHCl_3$); NMR δ 0·82 (d, *J* = 6 Hz, isopropyl), 2·06 (q, 2, *J* = 16 Hz, methylene between carboxyl and asymmetric carbon in R-group of 2), 3·53 and 3·64 (2 s, each 3, vinyl methoxyl and carbomethoxy), 5·01 (s, 1, vinyl), 5·82 (m, 2, methylenedioxy), 5·96 (d, 1, *J* = 10 Hz, methine on oxygen-bearing carbon), and 6·50 and 6·59 (2 s, each 1, aromatic); in DMSO a 1 H singlet at δ-4·73 disappeared when the sample was treated with D_2O (tertiary-OH); mass spectrum *m/e* (rel intensity) M^+ 515(5), 484(8), 314(12), 298(100), 266(27), 173(10), 159(3), 150(35), 141(3), 99(20) and 81(12).

Methyl 3-carbomethoxy-3-hydroxy-6-methylheptanoate (**9**) *and cephalotaxine* (**1**) *from transesterification of deoxyharringtonine* (**2**). Alkaloid **2** was placed in a vial and dried overnight at 78° in an Abderhalden tube with P_2O_5. The sample (0·092 g) was treated with 2·5 ml of 0·5 M NaOMe in MeOH, and the vial was capped and placed in a dry atmosphere at room temp for 5 hr. After AcOH aq (30 ml of 5%) was added, the solution was extracted with 30 ml of $CHCl_3$. After being washed with 5% AcOH and then Na_2CO_3 solutions, the $CHCl_3$ extract yielded 0·036 g of crude **9**, which was purified on an alumina column: IR 3595 (—OH), 1740 (ester C=O), 1100, 990 and 972 cm^{-1}; NMR δ 0·85 (d, 6, *J* = 6 Hz, isopropyl), 1·0–1·8 (m, 5, methylenes and methine of isovaleryl branch), 2·79 (q, 2, *J* = 16 Hz, methylene adjacent to carboxyl) and 3·64 and 3·77 (2 s, each 3, carbomethoxyl groups); mass spectrum *m/e* (rel intensity) 173(75), 141(10), 99(100) and 81(35). Extraction of the basified AcOH aq. phase (from isolation of **9** above) with $CHCl_3$ yielded cephalotaxine (**1**): IR, UV, NMR and $[\alpha]_D$ entirely consistent with those of pure cephalotaxine.[1a, 2]

Methyl 3-carbomethoxy-3-hydroxy-6-methylheptanoate (**9**). *Synthetic.* NaH (12·0 g), as a 55% dispersion in mineral oil, was placed in a 1-liter three-necked flask and was triturated five times with hexane. All manipulations were done in a dry N_2 atmosphere and all glassware heat-dried just before use. The NaH was suspended in anhyd ether (65 ml) and the suspension was stirred with a sealed constant-torque stirrer while 60·1 ml (0·5 mol) of diethyl carbonate was added. Then 28·5 g (0·25 mol) of 5-methyl-2-hexanone (**5**, from Chemical Samples Co., Columbus, Ohio) in ether (65 ml) was added slowly over 6 hr; the mixture was refluxed (with constant stirring) on a steam bath during this addition. The mixture stood overnight at room temp. Glacial AcOH (40 ml) was added slowly (1·5 hr) to the stirred, cold (ice bath) mixture; the resulting NaOAc was dissolved by addition of water. The acidic aqueous phase was extracted thoroughly with ether, and the extract washed to neutrality with dilute $NaHCO_3$ aq and then water. Crude **6**, 47·5 g, was obtained by removal of the ether *in vacuo*: IR 1710 and 1735 cm^{-1} (C=O); TLC with ether–hexane (10:90) revealed one spot, R_f 0·53.

The β-ketoester **6** (35·0 g) was stirred thoroughly at 0° with about 30 g of KCN. Conc HCl (40 ml) was added slowly (1·5 hr) from a dropping funnel and stirring at 0° was continued an additional 2·5 hr. The yield of crude cyanohydrin **7** obtained by ether extraction of this mixture was 38·2 g; IR 3630 cm^{-1} (—OH). Hydrolysis of this cyanohydrin ester (**7**) was achieved by treating it overnight at room temp with conc. HCl (200 ml) and heating it on a steam bath for 2 hr. Water (400 ml) was added and the mixture refluxed for 4 hr. Ether extraction of this acidic solution gave 41·3 g of crude 3-carboxy-3-hydroxy-6-methylheptanoic acid (**8**). Recrystallization from hexane–$CHCl_3$ (1:2·5) at −20° afforded 24·6 g of a white waxy solid: m.p.

78–80°; IR 3580 (—OH), 2500–3300 (free carboxyl groups) and 1720 cm^{-1} (C=O); mass spectrum *m/e* (rel intensity) no M^+, 159(83), 141(50), 99(100) and 81(32). Dimethyl ester **9** was prepared by treating **8** with CH_2N_2 or by refluxing **8** with 3% H_2SO_4 in MeOH followed by the usual ether extraction. This diester was a viscous liquid: IR 3595 (—OH), 1740 (ester C=O), 1100, 990 and 972 cm^{-1}; NMR δ 0·85 (d, 6, J = 6 Hz, isopropyl), 1·0–1·8 (m, 5, methylenes and methine of isovaleryl branch), 2·79 (q, 2, J = 16 Hz, methylene adjacent to carboxyl), 3·64 and 3·77 (2 s, each 3, carbomethoxyl groups); mass spectrum *m/e* (rel intensity) M^+ 232(1), 173(60), 141(10), 99(100) and 81(35).

Methyl 3-carboxy-3-hydroxy-6-methylheptanoate (**10**). A typical reaction involved treating 2·250 g of diacid **8** (dissolved in 4 ml of anhyd dioxane) with 0·84 ml of anhyd MeOH and two drops of conc H_2SO_4 for 7 to 14 days at room temp. The product was recovered by ether extraction. TLC analysis with ether–hexane–AcOH (55:45:2) revealed three spots due to diester **9**, R_f 0·70; half-ester **10**, R_f 0·38; and diacid **8**, R_f 0·06. A trace of half-ester **11** is observed at R_f 0·41. When separated by prep TLC with the same solvent system, this mixture yielded 1·365 g of **10**: IR 3595 (—OH), 2600–3300 (free carboxyl) and broad 1710–1740 cm^{-1} (both ester and free acid C=O); NMR δ 0·87 (d, 6, J = 6 Hz, isopropyl), 1·0–1·8 (m, 5, methylenes and methine of isovaleryl group), 2·85 (q, 2, J = 16 Hz, methylene adjacent to carbomethoxyl) and 3·68 (s, 3, carbomethoxyl); mass spectrum *m/e* (rel intensity) M^+ 218(9), 173(91), 159(34), 141(20), 99(100) and 81(31).

3-Carbomethoxy-3-hydroxy-6-methylheptanoic acid (**11**). A 2·00 g sample of diester **9** was dissolved in MeOH (75 ml), 88·0 ml of 0·098 N aqueous NaOH was added, and the mixture allowed to stand overnight at room temp. Acidification, followed by the usual ether extraction, afforded 1·78 g of white solid, **11**: m.p. 58–60°; TLC with ether–hexane–AcOH (55:45:2) revealed only one spot, R_f 0·40; IR 3595 (—OH), 2600–3300 (free carboxyl), 1735 (ester C=O) and 1720 (acid C=O); NMR δ 0·85 (d, 6, J = 6 Hz, isopropyl), 1·0–1·8 (m, 5, methylenes and methine of isovaleryl group), 2·83 (q, 2, J = 16 Hz, methylene adjacent to carboxyl), 3·76 (s, 3, carbomethoxyl); mass spectrum *m/e* (rel intensity) M^+ 218(9), 173(10), 159(82), 141(33), 99(100) and 81(35).

Pseudo-deoxyharringtonine (**3**). A 0·520 g sample of cephalotaxine (**1**), which had been dried in an Abderhalden for 24 hr at 78°, was dissolved in 3 ml of dry pyridine. The acid chloride of **11** (1·50 g), prepared with $SOCl_2$ at room temp, was added to the pyridine solution and the mixture stirred at room temperature for 3 hr. After the mixture stood overnight, it was treated with Na_2CO_3 solution and extracted with $CHCl_3$. This extract contained three major products—R_f 0·40, 0·45 and 0·52—by TLC with benzene–MeOH (9:1). Meticulous prep TLC with benzene–MeOH (85:15) resolved these three constituents into two fractions, one of which contained the constituent of R_f 0·52 (**4**, 24% of mixture). The second fraction (53%) contained the components of R_f 0·40 and 0·45: IR 3640 (—OH), 1745 (ester C=O), 1660 (trisubstituted double bond), 1485 (unassigned), 1040 (methyl ether) and 925–940 cm^{-1} (methylenedioxy); NMR δ 0·81 (d, 6, J = 6 Hz, isopropyl), 3·68 (s with a shoulder, 6, vinyl methoxyl and carbomethoxy), 5·02 (apparent d, 1, vinyl), 5·75 (pair of m, 0·5, methine on oxygen-bearing carbon), 5·86 (q, 0·5, J = 2 Hz, methine on oxygen-bearing carbon), 5·84 (s, 2, methylenedioxy) and 6·49, 6·50, 6·56 and 6·60 (4 s, 2 total, aromatic); mass spectrum *m/e* (rel intensity) M^+ 515(21), 484(12), 314(18), 298(100), 266(12), 173(4), 159(11), 150(16), 141(6), 99(23) and 81(20).

Dehydrated pseudo-deoxyharringtonine (**4**). Compound **4**, isolated above, was an amorphous solid: IR 1725 (α,β-unsaturated ester C=O), 1660 (trisubstituted double bond), 1040 and 925–940 cm^{-1} (methylenedioxy; NMR δ 0·87 (d, 6, J = 6 Hz, isopropyl), 3·67 and 3·69 (2 s, 3 each, vinyl methoxyl and carbomethoxyl), 5·03 (s, 1, vinyl), 5·80 (s, 2, methylenedioxy), 5·83 (finely split d, 1, J = 9 Hz, methine proton on oxygen-bearing carbon), 6·27 (s, 1, vinyl proton α- to ester grouping) and 6·51 and 6·56 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) M^+ 497(40), 466(8), 314(71), 298(100), 282(8), 266(17), 150(14) and 137(6).

Attempted synthesis of deoxyharringtonine (**2**). A 0·210 g sample of dry cephalotaxine (**1**) was dissolved in 2 ml of anhyd pyridine. The acid chloride of **10**, prepared by treating 0·530 g of the acid with $SOCl_2$ for 1·5 hr at room temp, was added dropwise to this solution of alkaloid. A drying tube was attached to the reaction vessel. The mixture was stirred 2 hr and was then allowed to stand at room temperature for 60 hr. Extraction of the basified (Na_2CO_3) solution with $CHCl_3$ afforded 0·470 g of an intractable, gummy black solid. TLC analysis with MeOH–$CHCl_3$ (15:85) revealed this mixture contained at least seven components none of which corresponded to deoxyharringtonine. Nearly identical results were obtained with a number of reaction times and various reactant ratios.

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