H-6), 2.20 (3 H, s, $C_4$-$OCOCH_3$), 2.02 (3 H, s, $C_8$-$OCOCH_3$), 1.52 (3 H, s, H-14), and 1.22 (3 H, d, $J$ = 6.0, H-15).

**Treatment of 14 with *m*-Chloroperbenzoic Acid. 1β-Hydroxyhelenalin Acetate (19) and 1α-Hydroxyhelenalin (11).** A solution of 14 (586 mg, 1.69 mmol) and *m*-chloroperbenzoic acid (400 mg) in $CHCl_3$ (50 ml) was allowed to stand at room temperature overnight. The reaction mixture was washed with 5% $Na_2SO_3$, 5% $NaHCO_3$, and $H_2O$, dried ($Na_2SO_4$), and evaporated *in vacuo* to give a yellowish oil. This was chromatographed on Florisil (Floridin Co., 100–200 A mesh, 1.3 × 30 cm) with benzene and $CHCl_3$ as the eluting solvents. The benzene fractions (50 ml) yielded, after evaporation, a pale yellowish oil (360 mg) which showed only a faster moving single spot on tlc. The nmr spectrum, however, indicated that this was a 1:1 mixture of two components. The subsequent $CHCl_3$ fractions (50 ml) gave, after evaporation, a colorless crystalline residue (160 mg, 29%) which corresponded to the slower moving spot on tlc. Recrystallization from $CHCl_3$–$Et_2O$ furnished **19**: mp 165–166°; nmr 7.35 (1 H, d, $J$ = 6.0, H-2), 6.40 (1 H, d, $J$ = 6.0, H-3), 6.22 (1 H, br s, H-13), 5.72 (1 H, br s, H-13), 5.25 (1 H, d, $J$ = 10.5, H-6), 4.37 (1 H, m, H-8), 3.70 (1 H, m, H-7), 2.10 (3 H, s, $C_6$-$OCOCH_3$), 1.21 (3 H, d, $J$ = 6.8, H-15), and 1.18 (3 H, s, H-14).

Attempted separation of the foregoing 1:1 mixture by silica gel column chromatography in $CHCl_3$ led to the isolation of two crystalline solids which were identified (tlc, mixture melting point, superimposable ir and nmr spectra) as **19** and **11**, respectively.

**Acknowledgment.** We thank Dr. David Rosenthal and Mr. Fred Williams of the Research Triangle Center for Mass Spectrometry for mass spectral data. This investigation was generously supported by U. S. Public Health Service Research Grant CA-12360 from the National Cancer Institute to K.H.L.

### References and Notes

(1) K. H. Lee, T. Ibuka, M. Kozuka, A. T. McPhail, and K. D. Onan, *Tetrahedron Lett.*, 2287 (1974) (part 10).
(2) K. H. Lee, *J. Pharm. Sci.*, **62**, 1028 (1973), and references cited therein.
(3) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2551 (1949).
(4) K. H. Lee, H. Furukawa, and E. S. Huang, *J. Med. Chem.*, **15**, 609 (1972).
(5) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2554 (1949).
(6) K. H. Lee, T. Ibuka, A. T. McPhail, K. D. Onan, T. A. Geissman, and T. G. Waddell, *Tetrahedron Lett.*, 1149 (1974).
(7) W. Herz, A. Romo de Vivar, J. Romo, and N. Viswanathan, *J. Amer. Chem. Soc.*, **85**, 19 (1963).
(8) K. H. Lee, H. Furukawa, S. H. Kim, and C. Piantadosi, *J. Pharm. Sci.*, **62**, 987 (1973).
(9) R. Adams and W. Herz, *J. Amer. Chem. Soc.*, **71**, 2546 (1949).
(10) K. H. Lee, R. Meck, C. Piantadosi, and E. S. Huang, *J. Med. Chem.*, **16**, 299 (1973).
(11) E. S. Huang, K. H. Lee, C. Piantadosi, T. A. Geissman, and J. S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972).
(12) K. H. Lee and I. H. Hall, unpublished results.

# Preparation and Antitumor Activity of a Rearranged Ester of Cephalotaxine†

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*Received April 29, 1974*

For further evaluation of structure–activity relationships among the *Cephalotaxus* alkaloids, a "rearranged" ester (**2b**) of cephalotaxine was prepared, one which is an isomer of deoxyharringtonine (**5a**). The parent alkaloid, cephalotaxine (**1a**), was allowed to react with thionyl chloride to replace its hydroxyl group with chlorine. The resulting chloro compound **1b**, on treatment with the silver salt of half ester **6**, yielded **2b** *via* an allylic rearrangement followed by further double bond migration. The new "rearranged" deoxyharringtonine isomer **2b** proved to be inactive in the P-388 lymphocytic leukemia system and thus further delineated the structural requirements for antitumor activity in this series of alkaloids.

A number of alkaloid esters from *Cephalotaxus harringtonia* have significant activity in experimental leukemia systems.[1,2] All of these active esters are derived from the same parent alkaloid, cephalotaxine (**1a**).[1,3,4] Although **1a** *per se* is inactive, there have been total syntheses of this unusual alkaloid by at least two groups,[5,6] as well as syntheses of the acyl moieties of three of its active esters—deoxyharringtonine (**5a**),[2,7a] harringtonine (**5b**),[7b] and isoharringtonine (**5d**).[7c] More recently, **5a** itself was prepared by partial synthesis from **1a**.[7d]

Variations in the acyl moiety of harringtonine and its active congeners naturally raise questions as to what structural features are essential for their antitumor activity. We demonstrated that mere introduction of an ester functionality does not transform **1a** into an active compound, since the acetate of **1a** is inactive (Table I). We also ascertained that the hindered *tert*-carboxyl group of the diacid moiety must be the one attached to cephalotaxine in order to preserve activity.[1,2] Accordingly, we have investigated another structural variation by synthesizing for bioassay purposes an isomer of deoxyharringtonine (**2b**) with the *tert*-carboxyl group joined to the alkaloid moiety but at a different position on the cyclopentene ring. The less accessible epimer **3** also was synthesized in small quantity.

Since the hydroxyl group of **1a** is in an allylic position, it seemed likely that we could replace it with chlorine and thus gain access to products of allylic rearrangement. This objective was realized by treating intermediate chloro compounds with the silver salt of the requisite half ester **6**. Rearranged esters were provided through a reaction that is formally SN2′-like in character, followed by a further double bond migration (see Scheme I).

When **1a** was treated with thionyl chloride in pyridine–ether solution, the main product was a chloro compound (**1b**) formed with inversion of configuration at C-3.‡ Reaction of **1b** with the silver salt of half ester **6** provided a new ester which was characterized as **2b** by nmr; its spectrum indicated that only one epimer was formed. Biological assay of "rearranged" ester **2b** revealed that its activity in the P-388 lymphocytic leukemia system, if any, is of a considerably lower order than that of harringtonine (**5b**), the

†Presented in part at the 166th National Meeting of the American Chemical Society, Chicago, Ill., August 26–31, 1973, MEDI 60.

‡Since the hydroxyl group of cephalotaxine has the *S* configuration,[8] the chloro compound that is derived by configurational inversion must have the *R* configuration. Accordingly, compound 1b is (3*R*)-3-chloro-3-deoxycephalotaxine. For convenience, we call this compound epicephalotaxyl chloride and its epimer (**1d**) cephalotaxyl chloride. Asada[9] recently reported an analogous chloro compound prepared by treating **1a** with phosphorus oxychloride, but he did not assign a configuration to this derivative.

first *Cephalotaxus* alkaloid identified as having antitumor activity[1] (see Table I).

Scheme I

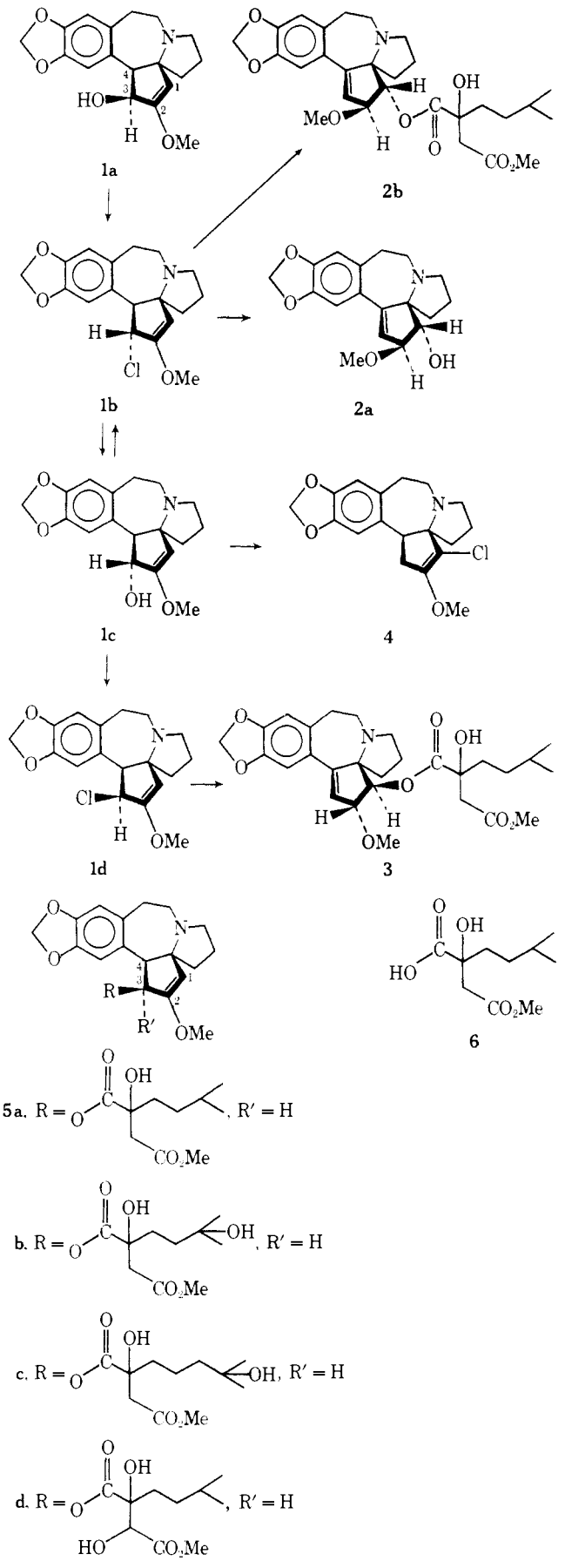


**Table I.** Assay of "Rearranged" Cephalotaxine Ester **2b** Compared with Harringtonine and Cephalotaxine Acetate in P-388 Lymphocytic Leukemia System[a]

| Compound | Dose, mg/kg | Survivors | Animal wt difference ($T-C$) | Survival time, days ($T/C$) | $T/C$, % |
|---|---|---|---|---|---|
| Rearranged ester **2b** | 29 | 4/4 | −0.3 | 11.5/11.0 | 105 |
| | 20 | 4/4 | −1.5 | 14.0/11.0 | 127 |
| | 10 | 4/4 | −0.2 | 11.0/11.0 | 100 |
| | 5 | 4/4 | −0.3 | 11.0/11.0 | 100 |
| Harringtonine **5b**[b] | 4 | 2/6 | −5.5 | 5.0/9.0 | |
| | 2 | 6/6 | −3.3 | 18.5/9.0 | 205 |
| | 1 | 6/6 | −2.3 | 36.5/9.0 | 405 |
| | 0.5 | 6/6 | −1.0 | 26.5/9.0 | 294 |
| Cephalotaxine acetate[c] | 80 | 6/6 | 0.4 | 11.5/11.0 | 105 |
| | 40 | 6/6 | 0.4 | 11.0/11.0 | 100 |
| | 20 | 6/6 | 0.4 | 11.0/11.0 | 100 |

[a] Compounds are considered active if $T/C \geq 125\%$. Assays were performed under the auspices of Drug Research and Development, National Cancer Institute, by intraperitoneal injection in mice. For general screening procedures and data interpretation, *cf.* R. T. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 1 (1972). [b] Previously published data for harringtonine[1] are cited for comparison. [c] Preparation of cephalotaxine acetate was described in ref 1.

Preparation of 3, epimeric with **2b** at C-1 and C-2, was less straightforward and was achieved only in low overall yield which precluded biological assay of this compound. Epicephalotaxine (**1c**) from natural sources was not available to us but was obtained by hydrolyzing chloride **1b** with hot water (66% yield).[§] A rearrangement product, **2a** (30%), was also produced. Reaction of **1c** with thionyl chloride in pyridine–ether afforded three identifiable products. Epicephalotaxyl chloride (**1b**) was formed with retention of configuration (49%) and cephalotaxyl chloride (**1d**) with inversion (17%); rearranged product **4** (34%) also was isolated. Ester **3** was obtained from **1d** by the same silver salt procedure that we applied to **1b**.

General features of nmr, ir, and mass spectra provide convincing evidence that **1b–d** possess the same cephalotaxine ring system without alterations or rearrangements. In addition, nmr spectra establish configurations of these compounds at C-3. For epicephalotaxine (**1c**) and the corresponding chloride **1b**, $J_{3,4} = 5$ Hz; in contrast, **1a** and **1d** have values of 9 and 10 Hz, respectively, for $J_{3,4}$.

In discussing reactions of thionyl chloride with allylic alcohols, Gould remarked that the intermediate chlorosulfite "may conceivably react by five mechanisms: SN1, SN2, SN2′, SNi, and SNi′." [11] Among the reactions just outlined, we have encountered examples consistent with most of these mechanistic types. The contrast in the steric course of reactions of **1a** and **1c** with thionyl chloride is striking and probably is determined by the steric environment of the epimeric reaction sites; molecular models of this cage-like ring system reveal that a substituent in the 3*S* configuration is much more hindered than one that is 3*R* (*i.e.*, has the epi configuration).

Apparently, rearranged esters **2b** and **3** are formed stereospecifically. The nmr spectra of the two are different,

[§] Epicephalotaxine was reported by Paudler and McKay[10] as a trace constituent of *C. fortunei* alkaloids and also as a minor product of lithium aluminum hydride reduction of cephalotaxinone. Their nmr data are in good agreement with our spectrum for 1c, but their optical rotation and melting point disagree with ours.

and each has values for $J_{1,2}$ and $J_{2,3}$ which according to the Karplus relationship[12,13] indicate a trans configuration[14] for substituents at C-1 and C-2. In an SN2′ reaction, one would expect that the attacking acyl group would enter cis to the leaving chloro group.[15] Accordingly, we tentatively assign the structures shown for **2b** and **3.** The initial allylic rearrangement must be followed by migration of the double bond to the 3,4 position, as evidenced by nmr spectra. However, examination of molecular models and uv spectra indicates that the 3,4 double bond is not conjugated (*i.e.*, coplanar) with the aromatic ring. Similar considerations may be applied to the structure of **2a,** a rearranged isomer of **1a** obtained as a by-product in hydrolysis of **1b.**

With harringtonine (**5b**) as our prototype, we can define certain structure–activity relationships for the *Cephalotaxus* alkaloids. The ester linkage appears to be essential, but insufficient. Insertion of an additional methylene group in the terminal portion of the acyl side chain has little effect since homoharringtonine (**5c**) has approximately the same activity as harringtonine.[1] On the other hand, removal of the hydroxyl group from the penultimate carbon of the acyl group (to give deoxyharringtonine, **5a**) reduces activity by about half.[1] Transfer of this same hydroxyl to give isoharringtonine (**5d**) lowers activity by almost one order of magnitude.[1] Attaching the "wrong" carboxyl group of the acyl moiety to cephalotaxine[1,2] or shifting the acyloxy group from C-3 to C-1 (present study) greatly reduces or abolishes antitumor activity. These observations point to a rather high specificity in structure–activity relationships of these alkaloids although many untested structural modifications are conceivable.

## Experimental Section

**General.** A Perkin-Elmer Model 137[&] spectrophotometer was used to record the ir spectra on 1% solutions in $CHCl_3$. Nmr spectra were obtained on $CDCl_3$ solutions with a Varian HA-100 spectrometer; chemical shifts are relative to internal TMS. Low-resolution mass spectral analyses were performed at 70 eV with a Du Pont CEC 21-492-1 spectrometer and high-resolution mass spectra with a Nuclide 12-90G spectrometer. Tlc analyses were done on Brinkman precoated 0.25-mm silica gel F-254 plates with solvents specified in the text and spots were visualized by staining with iodine vapor, by spraying with ethanolic Bromothymol Blue solution, or by charring with $H_2SO_4$–$K_2Cr_2O_7$. Preparative tlc was done on 1-mm silica gel G layers with Bromothymol Blue or dichlorofluorescein visualization. Unless specifically stated otherwise, reactions were carried out at room temperature. Molecular formulas were verified by high-resolution mass spectra because only small amounts of many of the natural product derivatives were available for analyses. Unless a melting point is indicated, all new compounds reported in this paper were persistently amorphous despite various attempts to crystallize them.[**]

**Epicephalotaxyl Chloride (1b).** Cephalotaxine (2.15 g, 6.8 mmol) and 0.60 ml (7.5 mmol) of dry pyridine were dissolved in 150 ml of dry ether, and the solution was cooled in an ice bath. Thionyl chloride (0.45 ml, *ca.* 6.5 mmol) in 5 ml of ether was added dropwise with vigorous stirring. The ice bath was removed and stirring was continued for 1 hr. Solvent was removed with a stream of nitrogen and a water aspirator. Additional $SOCl_2$ (1.5–2.0 ml) was added in four portions with aspiration for about 20 min between each addition. After all $SOCl_2$ had been added, the flask was heated for 0.5 min on a steam bath and was then evacuated for 1 hr at 40–45° (water bath). The black, tarry residue was slurried with 5% aqueous $Na_2CO_3$ solution and extracted with $CHCl_3$: yield, 88%. Tlc of this product with MeOH–$CHCl_3$ (15:85) revealed a major spot, $R_f$ 0.70, and a smaller spot of highly colored material, $R_f$ 0.81. Preparative tlc with MeOH–$CHCl_3$ (5:95) gave 65% of chloride **1b**; its ir spectrum is the same as that of cephalotaxine except the –OH absorption (3600 $cm^{-1}$) is absent and a sharp, unassigned band is observed at 1335 $cm^{-1}$; nmr $\delta$ 3.53 (d, 1, $J$ = 5 Hz, on C-4), 3.72 (s, 3, methoxyl), 4.76 (d, 1, $J$ = 5 Hz, on C-3), 4.98 (s, 1, vinyl), 5.87 (s, 2, methylenedioxy), and 6.62 and 6.68 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 333 (8) and 335 (3), 298 (100), 282 (6), 266 (20), 150 (9), 126 (4), and 83 (4). Found: $M^+$, 333.112. $C_{18}H_{20}NO_3Cl$ requires 333.113.

**Hydrolysis of Epicephalotaxyl Chloride (1b) with Hot Water.** Hydrolysis of **1b** can be accomplished by refluxing with water for 1 hr. Addition of $Na_2CO_3$ solution and extraction with $CHCl_3$ gives quantitative recovery of a product containing 66% of epicephalotaxine (**1c**) [as isolated by preparative tlc with MeOH–$CHCl_3$ (10:90)]: mp 201–203° (from ether); ir and tlc characteristics are essentially the same as those of cephalotaxine; $[\alpha]^{26}D$ −42.5° (*c* 0.32, $CHCl_3$); nmr $\delta$ 3.09 (d, 1, $J$ = 5 Hz, on C-4), 3.67 (s, 3, methoxyl), 4.65 (d, 1, $J$ = 5 Hz, on C-3), 4.82 (s, 1, vinyl), 5.85 (s, 2, methylenedioxy), and 6.60 and 6.67 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 315 (100), 300 (57), 298 (58), 284 (47), 272 (13), 254 (13), 214 (11), 166 (43), 150 (21), and 137 (31). Found: $M^+$, 315.147. $C_{18}H_{21}NO_4$ requires 315.147.

A rearrangement product, **2a,** is also formed (30%) by hydrolysis of **1b.** The ir spectrum of **2a** showed only minor variations from that of cephalotaxine; on tlc with MeOH–$CHCl_3$, it migrated slightly farther than cephalotaxine; nmr $\delta$ 3.62 (s, 3, methoxyl), 3.98 (dd, 1, $J_{1,2}$ = 3 Hz and $J_{2,3}$ = 2 Hz, on C-2), 4.51 (d, 1, $J$ = 3 Hz, on C-1), 4.72 (d, 1, $J$ = 2 Hz, on C-3), 5.86 (s, 2, methylenedioxy), and 6.57 and 6.60 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 315 (79), 300 (62), 298 (18), 284 (23), 228 (100), 216 (17), 173 (4), and 150 (4). Found: $M^+$, 315.145; $C_{18}H_{21}NO_4$ requires 315.147.

**Cephalotaxyl Chloride (1d).** A representative reaction used 0.250 g (0.8 mmol) of **1c** dissolved in ether and cooled in an ice bath. Anhydrous pyridine (0.07 ml, 0.9 mmol) and thionyl chloride (0.06 ml, 0.8 mmol) were added as previously described, and the product was isolated by $CHCl_3$ extraction: yield, 85%. Tlc with MeOH–$CHCl_3$ (15:85) revealed three major components, $R_f$ 0.52, 0.68, and 0.82, which were isolated by preparative tlc using MeOH–$CHCl_3$ (8:92). The predominant product (49%) had $R_f$ 0.68 and was identical in all respects with **1b.** The spot with $R_f$ 0.52 was **1d:** yield, 17%. Its ir spectrum was the same as that of **1b;** nmr $\delta$ 3.72 (s, 3, methoxyl), 3.82 (d, 1, $J$ = 10 Hz, on C-4), 5.02 (s, 1, vinyl), 5.07 (d, 1, $J$ = 10 Hz, on C-3), 5.87 (s, 2, methylenedioxy), and 6.55 and 6.62 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 333 (18) and 335 (7), 298 (100), 282 (13), 266 (28), 199 (16), 150 (25), 126 (25), and 83 (26).

The third product, $R_f$ 0.82 (34%), from chlorination of **1c** was shown to be compound **4.** Ir analysis revealed that the 1645-$cm^{-1}$ band (trisubstituted double bond) of **1d** and **1b** shifted to 1665 $cm^{-1}$ and also that a minor, unassigned band at 982 $cm^{-1}$ was missing; nmr $\delta$ 2.1–3.4 (overlapping multiplets), 3.68 (s, 3, methoxyl), 5.84 (s, 2, methylenedioxy), and 6.54 and 6.60 (2 s, 1 each, aromatic), vinyl proton absent; mass spectrum *m/e* (rel intensity) $M^+$ 333 (60) and 335 (14), 318 (29), 298 (47), 282 (17), 186 (33), 184 (100), 176 (24), 139 (19), 115 (23), and 91 (18). Found: $M^+$, 333.112; $C_{18}H_{20}NO_3Cl$ requires 333.113.

**Reaction of Cephalotaxyl Chloride (1d) and Epicephalotaxyl Chloride (1b) with the Silver Salt of Methyl 3-Carboxy-3-hydroxy-6-methylheptanoate (6).** This synthetic half ester (**6,** previously synthesized[2]) was converted to its anhydrous silver salt. The salt (0.173 g, 0.41 mmol) was then suspended in dry benzene, 0.166 g (0.50 mmol) of **1d** in benzene was added, and the mixture was stirred 19 hr. The residue remaining after most of the solvent had been removed was slurried with $CHCl_3$. Aqueous $Na_2CO_3$ solution (5%) was added and the $CHCl_3$ extraction completed: yield, 58%. From this mixture (mostly unreacted alkaloid) was isolated 0.030 g (20%) of rearranged cephalotaxine ester **3:** ir 3600 (–OH), 1740 (ester –C=O), 1660 (trisubstituted olefin), 1040 (methoxyl), and 930 $cm^{-1}$ (methylenedioxy); nmr $\delta$ 0.86 (d, 6, $J$ = 6 Hz, isopropyl), 3.57 (s, 3, allylic methoxyl), 3.64 and 3.66 (2 s,[††] 3, carbomethoxy), 4.00 (2 dd,[††] 1, $J_{1,2}$ = 2 Hz and $J_{2,3}$ = 2 Hz, on C-2), 4.65 (d, 1, $J$ = 2 Hz, vinyl), 5.88 (s, 2, methylenedioxy), 6.07 [distorted t (two overlapping doublets[††]), 1, $J$ = 2 Hz, on C-1], and 6.59 and

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

[**]Cephalotaxine derivatives, both synthetic and natural, are notoriously resistant to crystallization and after many attempts even the natural harringtonines have been obtained only in amorphous states.

[††]Esters **2b** and **3,** prepared by the reaction of the silver salt of racemic **6** with chlorides **1b** and **1d,** respectively, are both comprised of two diastereomers; certain protons in these pairs of diastereomers generate nmr signals with slightly different chemical shifts, although their multiplicities and coupling constants are indistinguishable. This effect increases the apparent complexity of the signals indicated.

6.62 (2 s, 1 each, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 515 (22), 484 (4), 314 (20), 298 (100), 282 (12), 266 (10), 228 (20), 173 (24), 150 (12), and 99 (40). Found: $M^+$, 515.245; $C_{28}H_{37}NO_8$ requires 515.252.

Compound **1b** was treated with the silver salt of **6** as described: yield of **2b** by preparative tlc, 56%. Its ir spectrum was identical with that of **3**: uv $\lambda_{max}$ (EtOH) 292 nm ($\epsilon$ 4895); $[\alpha]^{26}D$ −155.7° (*c* 0.42, $CHCl_3$); nmr $\delta$ 0.86 (d, 6, $J$ = 6 Hz, isopropyl), 3.55 (s with shoulder,†† 3, allylic methoxyl), 3.62 and 3.65 (2 s,†† 3, carbomethoxyl), 3.95 (2 dd,†† 1, $J_{1,2}$ = 2 Hz, $J_{2,3}$ = 2 Hz, on C-2), 4.63 (d, 1, $J$ = 2 Hz, vinyl), 5.85 (s, 2, methylenedioxy), 6.00 and 6.04 (2 d,†† 1, $J$ = 2 Hz, on C-1), and 6.58 (br s, 2, aromatic); mass spectrum *m/e* (rel intensity) $M^+$ 515 (40), 484 (10), 314 (36), 298 (100), 282 (9), 266 (7), 228 (38), 173 (16), 150 (8), and 99 (24). Found: $M^+$, 515.251; $C_{28}H_{37}NO_8$ requires 515.252.

**Acknowledgments.** We thank Dr. D. Weisleder for nmr spectra, Mr. R. Kleiman, Dr. W. K. Rohwedder, and Mr. R. D. Plattner for mass spectra, and Dr. W. H. Tallent, Dr. K. D. Carlson, and Dr. M. D. Grove for helpful discussions.

## References

(1) R. G. Powell, D. Weisleder, and C. R. Smith, Jr., *J. Pharm. Sci.*, **61**, 1227 (1972).
(2) K. L. Mikolajczak, R. G. Powell, and C. R. Smith, Jr., *Tetrahedron*, **28**, 1995 (1972).
(3) R. G. Powell, D. Weisleder, C. R. Smith, Jr., and I. A. Wolff, *Tetrahedron Lett.*, 4081 (1969).
(4) R. G. Powell, D. Weisleder, C. R. Smith, Jr., and W. K. Rohwedder, *Tetrahedron Lett.*, 815 (1970).
(5) J. Auerbach and S. M. Weinreb, *J. Amer. Chem. Soc.*, **94**, 7172 (1972).
(6) M. F. Semmelhack, B. P. Chong, and L. D. Jones, *J. Amer. Chem. Soc.*, **94**, 8629 (1972).
(7) (a) J. Auerbach, T. Ipaktchi, and S. M. Weinreb, *Tetrahedron Lett.*, 4561 (1973); (b) T. R. Kelly, J. C. McKenna, and P. A. Christenson, *ibid.*, 3501 (1973); (c) T. Ipaktchi and S. M. Weinreb, *ibid.*, 3895 (1973); (d) K. L. Mikolajczak, C. R. Smith, Jr., D. Weisleder, T. R. Kelley, J. C. McKenna, and P. A. Christenson, *ibid.*, 283 (1974).
(8) S. K. Arora, R. B. Bates, R. A. Grady, and R. G. Powell, *J. Org. Chem.*, **39**, 1269 (1974).
(9) S. Asada, *Yakugaku Zasshi*, **93**, 916 (1973).
(10) W. W. Paudler and J. McKay, *J. Org. Chem.*, **38**, 2110 (1973).
(11) E. S. Gould in "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, New York, N.Y., 1959, pp 294–296.
(12) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(13) R. R. Fraser, M. Kaufman, P. Morand, and G. Govil, *Can. J. Chem.*, **47**, 403 (1969).
(14) F. G. Cocu, G. Wolczunowicz, L. Bors, and Th. Posternak, *Helv. Chim. Acta*, **53**, 739 (1970).
(15) (a) W. G. Young, I. D. Webb, and H. L. Goering, *J. Amer. Chem. Soc.*, **73**, 1076 (1951); (b) G. Stork and W. N. White, *ibid.*, **78**, 4609 (1956); (c) J. Hine in "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N.Y., 1962, pp 152–154; (d) W. Drenth, *Recl. Trav. Chim. Pays-Bas*, **86**, 318 (1967).

# Preparation and Antileukemic Activity of Some Alkoxybenzo[*c*]phenanthridinium Salts and Corresponding Dihydro Derivatives[1]

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Salts of 2,3,8,9-tetrasubstituted alkoxy-, hydroxy-, and acetoxybenzo[c]phenanthridines as well as the corresponding 6-methoxy-5,6-dihydrobenzo[c]phenanthridines were prepared from appropriate chalcones through the tetralone and the 4b,10b,11,12-tetrahydrobenzo[c]phenanthridine intermediates. Complete O-demethylation of the tetramethoxybenzophenanthridine was achieved by fusion with pyridine hydrochloride at elevated temperature. The title compounds are active against leukemias L1210 and P388 in mice and some are curative against Lewis lung carcinoma. The importance of the nature of the environment about the nitrogen atom of these compounds and the substituents is discussed. 3,4-Dimethoxy-3′,4′-methylenedioxychalcone possesses activity against leukemia P388.

A number of alkoxybenzophenanthridine alkaloids have demonstrated interesting biological and pharmacological activities. Among these, nitidine chloride[2-4] (**1a**, X = Cl) and 6-methoxy-5,6-dihydronitidine[3,4] (**2a**), isolated from *Fagara macrophylla*, were shown to be highly cytotoxic, displayed antileukemic activity in both leukemia L1210 and P388 systems in mice, and inhibited Lewis lung carcinoma. Fagaronine[5] (**1b**), isolated from *Fagara zanthoxyloides* Lam., showed good activity against leukemia P388. This paper presents the preparation and antileukemic activity of several 2,3,8,9-tetrasubstituted benzophenanthridinium salts related to nitidine and allied compounds.[4,6]

**Chemistry.** The general synthetic route leading to the substituted benzo[*c*]phenanthridine ring system **5** follows essentially the same procedure as that used for the synthesis of nitidine[4] and allonitidine.[6] The overall yields of the nine-step synthesis of benzo[*c*]phenanthridinium salts **1** from the appropriate acetophenones through chalcones **3** and the tetrahydro intermediates **4** were 9–15%. Treatment of **1** in cold $NH_4OH$ followed by heating the intermediate with $CH_3OH$ afforded the methoxyl derivatives of dihydrobenzophenanthridine (**2**).

It has been postulated that the alkoxyl functions on compounds **1** and **2** may have to be dealkylated *in vivo* for necessary biological action. Consequently, preparation of the tetrahydroxy (**1e**) and the tetraacetoxy (**1f**) analogs of nitidine was conducted. Demethylation of 2,3,8,9-tetramethoxybenzo[*c*]phenanthridine (**5d**) was studied with a variety of known demethylating agents including 30% HBr in AcOH,[7,8] $BBr_3$ in $CHCl_3$,[9,10] and pyridine hydrochloride.[11,12] None of these agents under ordinary reaction conditions yielded completely demethylated product. For example, refluxing a mixture of **5d** and pyridine hydrochloride in nitrobenzene for extended periods only resulted in cleavage of two methoxy groups. However, when the aforementioned reactants were fused at 280° for 3 hr, complete demethylation took place with simultaneous N-methylation, and the yield of compound **1e** (X = Cl), complexed with 2 equiv of pyridine hydrochloride, was practically quantitative.

Acetylation of **1e** was accomplished in over 80% yield by heating the compound with a mixture of $Ac_2O$ and pyridine for 2 hr at 145–150°. The product, **5f**, could not be methylated under the usual reaction conditions used for