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Antitumor Alkaloids from *Cephalotaxus harringtonia*: Structure and Activity

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Abstract □ Cephalotaxine and several of its esters were isolated from *Cephalotaxus harringtonia* K. Koch var. *harringtonia*. Although cephalotaxine is inactive, harringtonine, isoharringtonine, homoharringtonine, and deoxyharringtonine have shown significant activity against experimental P388 leukemia and against L-1210 leukemia in mice.

Keyphrases □ *Cephalotaxus harringtonia* alkaloids—structure, antitumor activity □ Harringtonine, isoharringtonine, homoharringtonine, deoxyharringtonine—antitumor activity □ Antitumor alkaloids from *Cephalotaxus harringtonia*—structure, activity □ NMR spectroscopy—identification, *Cephalotaxus* alkaloids

In a search for tumor inhibitors of plant origin, an alcoholic extract of the seed of *Cephalotaxus harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi[1] showed activity against lymphoid leukemia L-1210 and P388 leukemia in mice[2]. Subsequent fractionation of the seed extract and of an extract obtained from *C. harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata (entire plants) revealed four alkaloids with significant antitumor activity (1). The active *Cephalotaxus* alkaloids are esters of cephalotaxine (I); these include harringtonine (II), isoharringtonine (III), homoharringtonine (IV), and deoxyharringtonine (V).

DISCUSSION

Paudler *et al.* (2) first isolated cephalotaxine, and their work indicated that two partial structures were possible. Subsequent investigations by other workers, using a combination of NMR (3) and X-ray crystallographic (4) techniques, revealed that cephalotaxine has the structure indicated here (I). We have now characterized the active antitumor alkaloids II–IV and report test data for these and several related alkaloids[3].

The NMR spectra of alkaloids II–V yielded initial evidence that these compounds are esters of cephalotaxine. This conclusion was based primarily on a comparison of their NMR spectra with the NMR spectra of cephalotaxine and acetylcephalotaxine (VII, Table I). If one disregards signals attributed to the R group, the NMR spectra of the cephalotaxine esters are nearly identical. The number and nature of free hydroxyl groups in alkaloids I–IV were indicated by NMR spectra of dimethyl sulfoxide-d_6 solutions before and after deuterium oxide exchange (5). In the mass spectra of these alkaloids, the strongest ion (base peak) is at m/e 298 ($C_{18}H_{20}NO_3$). This ion corresponds to cephalotaxine minus the appropriate R group.

Transesterification of alkaloids II–IV (sodium methoxide–methanol or sodium ethoxide–ethanol) gives alkaloid I, along with the corresponding dimethyl or diethyl esters (VIII–X or XII–XIV). Structures of Compounds VIII–X were deduced from NMR and mass spectral data.

Significant features of the NMR spectra of dimethyl esters VIII and X (see *Experimental* section for chemical shift assignments) are

[1] *Cephalotaxus* plant materials were received from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., under a program developed with USDA by Drug Research and Development, National Cancer Institute (formerly the Cancer Chemotherapy National Service Center).

[2] Assays performed under Drug Research and Development auspices. Procedures are described in *Cancer Chemother. Rep.*, **25**, 1(1962).

[3] The previously used numbering for the cephalotaxine ring system was revised. The revised numbering corresponds closely to that commonly used for the erythrina series of alkaloids.

Table I—NMR Data for Cephalotaxine and Some Cephalotaxine Esters[a]

Proton(s)	Alkaloid I	II	III	IV	V	VII
H-1	s 4.89	s 5.07	s 5.06	s 5.05	s 5.04	s 5.05
H-3[b]	d 4.70	d 5.99	d 6.02	d 5.99	d 5.98	d 5.80
H-4[b]	d 3.63	d 3.77	d 3.77	d 3.77	d 3.76	d 3.77
H-14	s 6.65	s 6.61	s 6.64	s 6.61	s 6.60	s 6.59
H-17	s 6.61	s 6.54	s 6.53	s 6.54	s 6.52	s 6.57
Aryl—OCH_2O—	s 5.86	s 5.85	m 5.82	s 5.85	m 5.84	s 5.85
Vinyl—OCH_3	s 3.70	s 3.68	s 3.67	s 3.67	s 3.66	s 3.71
$-\overset{O}{\overset{\|}{C}}-OCH_3$	—	s 3.57	s 3.60	s 3.58	s 3.54	—
$-C(OH)(CH_3)_2$	—	s 1.17	—	s 1.18	—	—
$-CH(CH_3)_2$	—	—	d 0.86	—	d 0.84	—
$-CCH_3C-$[c]	—	q 2.10	—	q 2.10	q 2.07	—
$-O\overset{O}{\overset{\|}{C}}CH_3$	—	—	—	—	—	s 1.58

[a] Measured in chloroform with a Varian HA-100 spectrometer. Chemical shifts (δ) are expressed in parts per million from tetramethylsilane. [b] In each of these alkaloids, protons H-3 and H-4 are coupled (J = 9.5 Hz.), and long-range coupling (J = 0.5 Hz.) is observed between protons H-1 and H-3. [c] These protons show strong geminal coupling (J = 16 Hz.).

as follows. Both show the presence of two equivalent methyl groups, two different carbomethoxyl groups, two tertiary hydroxyl groups, and an isolated methylene group. Dimethyl ester IX differs from VIII and X in that the two methyl groups appear as a doublet, characteristic of an isopropyl group; a singlet due to an isolated proton on a carbon bearing a secondary hydroxyl is present; and only one tertiary hydroxyl is apparent. The remaining protons appear as complex signals in the δ 1.20–1.90 region of VIII and X and in the δ 1.10–2.20 region of IX. The absence of a methoxyl resonance in the spectra of diethyl esters XII–XIV confirms the assignment of the carbomethoxyl signals near δ 3.60 for the parent alkaloids.

Data for significant fragments in the mass spectra are given in the *Experimental* section. Although molecular ions were absent in the mass spectra of esters VIII and X, an $M^+ + 1$ ion was detected at *m/e* 263 with an excessive sample pressure of X (6). That VIII and X are homologs was indicated by the appearance of parallel series of peaks differing by 14 mass units in the spectra of the two esters. The different number of methylene groups in the main carbon chains also led to some differences in the fragmentation patterns of these two compounds. On the other hand, the different location of one hydroxyl group in IX as compared to VIII leads to a completely distinct spectrum for IX. No molecular ion was apparent in its spectrum, and the peak of highest mass appeared at *m/e* 189 ($M^+ - COOCH_3$).

Final proof for the structures of esters VIII–X was recently obtained from synthetic studies directed toward the characterization of alkaloid V. This work resulted in the synthesis of dimethyl ester XI and also of pseudo-deoxyharringtonine (VI) (7). Synthetic VI differs from alkaloid V in that the alternative carboxyl group is esterified to cephalotaxine. This conclusion is based on the NMR spectra; the carbomethoxyl signal appears at δ 3.53 in the spectrum of V and at δ 3.70 in the spectrum of VI. The primary carbomethoxyl of dimethyl ester XI was assigned to the signal at δ 3.64, and the tertiary carbomethoxyl was assigned to the downfield signal at δ 3.77 (similar shifts for primary and tertiary carbomethoxyl groups are apparent in the spectrum of trimethylcitrate). From their position in the NMR spectra, the carbomethoxyl groups of alkaloids II–IV are by analogy considered to be primary.

Several *Cephalotaxus* alkaloids were tested against L-1210 or P388 leukemia (or both) (Tables II and III). From the relative survival times of treated (T) and control (C) animals (*i.e.*, T/C, %), it is evident that alkaloids II–IV show marginal activity against L-1210 and that I and VII are inactive.

Much greater activity is shown by alkaloids II–V against P388 leukemia, and this activity is apparent over a wide range of dosage levels. Alkaloids II, IV, and V exhibit the greatest activity at a 1–2 mg./kg. dosage level, whereas III has greatest activity at about 10 times this level. Since these compounds differ only in the ester (R) group, slight modification of this moiety significantly affects antitumor activity. The importance of the R group is further emphasized because cephalotaxine (I) and acetylcephalotaxine (VII) are inactive. On the other hand, pseudo-deoxyharringtonine (VI) gave a T/C of 122 at the highest dose level tested (40 mg./kg. against P388 leukemia) with no apparent toxic effects. Alkaloid VI has not yet been tested at higher levels owing to lack of material. Synthesis of cephalotaxine esters having other R groups may lead to compounds having even more desirable antitumor properties than II–V.

EXPERIMENTAL[4]

A typical isolation procedure for the *Cephalotaxus* alkaloids was described elsewhere (8). All attempts to crystallize alkaloids II–IV (from methanol, ether, benzene, petroleum ether, or mixtures of these solvents) failed; but because each gave a single spot on thin-layer chromatograms and a clean NMR spectrum, no contaminants were present in significant quantities.

Cephalotaxine (I)—Cephalotaxine was crystallized by slow evaporation of an ether solution in a loosely capped vial, m.p. 134–136°; $[\alpha]_D$ −189° (c 0.51 in chloroform), $[\alpha]_D$ −209° (c 0.23 in ethanol); λ_{max}: 290 (log ϵ 3.64), λ_{min}: 260 (log ϵ 2.75), λ_{max}: 238 nm. (log ϵ 3.56); ν_{max}: 3680, 1650, 1490, 1040, and 934 cm.$^{-1}$. The mass spectrum of I yielded prominent ions at *m/e* 315 (M^+, 100%), 300 (54), 298 (57), 284 (67), 272 (17), 254 (15), 214 (19), 166 (36), 150 (23), 137 (26), and 115 (16).

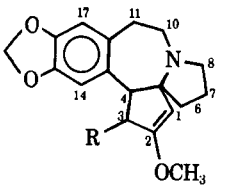
Anal.—Calc. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.72; N, 4.44. Found: C, 68.71; H, 7.04; N, 4.32.

The NMR spectrum of cephalotaxine is given in Table I. In dimethyl sulfoxide-d_6 solution, cephalotaxine exhibits a one-proton doublet, at δ 4.78, which is coupled to H-3 (q, δ 4.51). After exchange in deuterium oxide, only the H-3 signal is apparent (d, δ 4.51).

An authentic sample of cephalotaxine (2), recrystallized from ether, gave m.p. 135–136°, $[\alpha]_D$ −211° (c 0.04 in ethanol). The NMR, IR, and UV spectra of this material, as well as the corresponding spectra of all cephalotaxine samples isolated in this study, were indistinguishable.

Harringtonine (II)—Harringtonine was obtained as a white amorphous solid by evaporation of an ether solution under vacuum, $[\alpha]_D$ −106° (c 0.13 in chloroform); λ_{max}: 291 (log ϵ 3.61), λ_{min}: 261 nm. (log ϵ 2.74); ν_{max}: 3600, 1740, 1650, 1480, 1115, 1080, and 930 cm.$^{-1}$. The mass spectrum of II showed peaks at *m/e* 531 (M^+, 17%),

[4] Melting points were determined on a Fisher-Johns block and are uncorrected. IR spectra were measured in chloroform solutions on a Perkin-Elmer model 137 instrument, and UV spectra were obtained in absolute ethanol on a Beckman DK-2A spectrophotometer. Optical rotations were determined on a Cary model 60 recording spectropolarimeter at 26° in 0.5-dm. cells. Mass spectral analyses were performed with a Nuclide 12-90G spectrometer. Empirical formulas determined by high resolution are given in parentheses along with relative intensities. NMR spectra were measured with a Varian HA-100 in $CDCl_3$ solution, unless otherwise specified.



I: R = —OH

II: R = $CH_3C(CH_2)_2CCH_2CO_2CH_3$ (OH, OH; CH_3; CO_2^-)

III: R = $CH_3CH(CH_2)_2C{-}CHCO_2CH_3$ (OHOH; CH_3; CO_2^-)

IV: R = $CH_3C(CH_2)_3CCH_2CO_2CH_3$ (OH, OH; CH_3; CO_2^-)

V: R = $CH_3CH(CH_2)_2C{-}CH_2CO_2CH_3$ (OH; CH_3; CO_2^-)

VI: R = $CH_3CH(CH_2)_2CCH_2CO_2^-$ (OH; CH_3; CO_2CH_3)

VII: R = $CH_3CO_2^-$

R

$CH_3C(CH_2)_2CCH_2CO_2R''$ (OH, OH; CH_3; CO_2R') VIII: R′ = R″ = CH_3; XII: R′ = R″ = C_2H_5

$CH_3CH(CH_2)_2C{-}CHCO_2R''$ (OH OH; CH_3; CO_2R') IX: R′ = R″ = CH_3; XIII: R′ = R″ = C_2H_5

$CH_3C(CH_2)_3CCH_2CO_2R''$ (OH, OH; CH_3; CO_2R') X: R′ = R″ = CH_3; XIV: R′ = R″ = C_2H_5

$CH_3CH(CH_2)_2CCH_2CO_2R''$ (OH; CH_3; CO_2R') XI: R′ = R″ = CH_3

516 (1), 500 (4), 315 (8), 314 (7), 298 (100), 284 (7), 282 (6), 266 (13), 150 (11), and 116 (7). Found: M^+, *m/e* 531.246; $C_{28}H_{37}NO_9$ requires 531.247.

Anal.—Calc. for $C_{28}H_{37}NO_9$: C, 63.26; H, 7.02; N, 2.63; O, 27.1; OCH_3, 11.67. Found: C, 62.30; H, 7.20; N, 2.46; OCH_3, 12.86[5].

Data from the NMR spectrum of harringtonine are given in Table I. In dimethyl sulfoxide-d_6, II exhibits a pair of one-proton singlets at δ 4.74 and 3.99. Both signals are absent after exchange with deuterium oxide.

Isoharringtonine (III)—Evaporation of an ether solution, under vacuum, gave isoharringtonine as a white amorphous solid, $[\alpha]_D$ −93° (c 0.41 in chloroform); λ_{max}: 290 (log ε 3.60), λ_{min}: 261 nm. (log ε 2.72); ν_{max}: 3600, 1740, 1650, 1480, 1080, and 930 $cm.^{-1}$. The mass spectrum of III contains ions at *m/e* 531 (M^+, 14%), 516 (1), 500 (3), 442 (2), 315 (6), 314 (7), 298 (100), 284 (6), 282 (8), 266 (13), 178 (7), 150 (17), 99 (9), 90 (9), and 81 (9). Found: *m/e* 531.250; $C_{28}H_{37}NO_9$ requires 531.247. NMR data are given in Table I. In dimethyl sulfoxide-d_6, isoharringtonine gave a one-proton singlet at δ 4.54 and a one-proton doublet at δ 4.94. Both signals are absent after exchange with deuterium oxide.

Anal.—Calc. for $C_{28}H_{37}NO_9$: C, 63.26; H, 7.02; N, 2.63. Found: C, 63.17; H, 7.06; N, 2.58.

Homoharringtonine (IV)—Evaporation of an ether solution, under vacuum, gave homoharringtonine as an amorphous white solid, $[\alpha]_D$ −119° (c 0.45 in chloroform); λ_{max}: 290 (log ε 3.62), λ_{min}: 261 nm. (log ε 2.76); ν_{max}: 3580, 1740, 1650, 1480, 1070, and 928 $cm.^{-1}$. The mass spectrum of IV gave ions at *m/e* 545 (M^+, 14%), 530 (1), 514 (3), 315 (4), 314 (6), 298 (100), 284 (4), 282 (6), 266 (12), 205, (3), 150 (11), and 116 (5). Found: *m/e* 545.255; $C_{29}H_{39}NO_9$ requires 545.262. Data from the NMR spectrum of homoharringtonine will be found in Table I. In dimethyl sulfoxide-d_6, IV gave a pair of one-proton singlets at δ 4.75 and 3.97. Both signals are absent after exchange with deuterium oxide.

Anal.—Calc. for $C_{29}H_{39}NO_9$: C, 63.84; H, 7.20; N, 2.57. Found: C, 63.67; H, 7.24; N, 2.46.

[5] Although this analysis is not within normally accepted limits, the empirical formula ($C_{28}H_{37}NO_9$) is established by the high-resolution mass spectrum. In addition, VIII from transesterification of II gave satisfactory elemental analysis, and I was identical to an authentic sample of cephalotaxine.

Table II—Activity of Some *Cephalotaxus* Alkaloids against Lymphoid Leukemia L-1210[a]

Alkaloid	Dose, mg./kg.	Survivors	Animal Weight Difference (T − C)	Survival Time, Days (T/C)	T/C, %
I	220	6/6	−1.2	9.8/9.6	102
	110	6/6	0.2	10.3/9.6	107
	55	6/6	0.5	9.5/9.6	98
II	4.00	1/6	−4.1	0.0/9.1	—
	2.00	6/6	−2.4	12.5/9.1	137
	1.00	6/6	−1.2	12.3/9.1	135
	0.50	6/6	−1.0	12.0/9.1	131
III	15.0	6/6	−3.5	10.0/9.1	109
	7.50	6/6	−1.3	11.5/9.1	126
	3.75	6/6	−0.5	11.3/9.1	124
	1.87	6/6	−1.1	10.0/9.1	109
IV	2.00	6/6	−3.0	9.2/9.1	101
	1.00	6/6	−1.4	13.0/9.1	142
	0.50	6/6	−0.8	11.0/9.1	120
	0.25	6/6	−0.5	11.2/9.1	123
VII	100	6/6	−0.6	9.8/9.6	102
	50	6/6	0.0	10.2/9.6	106
	25	6/6	−0.3	10.2/9.6	106

[a] Data presented are representative of results from several assays with different samples of each alkaloid (Footnote 2). Materials are considered active if the survival time of animals treated (T) with them is ≥ 125% of that of the controls (C) (*i.e.*, T/C ≥ 125%).

Table III—Activity of Some *Cephalotaxus* Alkaloids against P388 Lymphocytic Leukemia[a]

Alkaloid	Dose, mg./kg.	Survivors	Animal Weight Difference (T − C)	Survival Time, Days (T/C)	T/C, %
II	4.00	2/6	−5.5	5.0/9.0	—
	2.00	6/6	−3.3	18.5/9.0	205
	1.00	6/6	−2.3	36.5/9.0	405
	0.50	6/6	−1.0	26.5/9.0	294
III	15.0	6/6	−4.3	9.5/9.0	105
	7.50	6/6	−3.0	24.5/9.0	272
	3.75	6/6	−2.8	15.5/9.0	172
	1.87	5/6	−1.3	13.5/9.0	150
IV	2.00	6/6	−3.8	7.5/9.0	...
	1.00	6/6	−2.8	30.5/9.0	338
	0.50	6/6	−1.8	24.5/9.0	272
	0.25	6/6	−2.2	22.0/9.0	244
V	4.00	6/6	−3.4	14.0/10.0	140
	2.00	6/6	−3.3	18.0/10.0	180
	1.00	6/6	−2.4	15.5/10.0	155
	0.50	6/6	−1.2	14.5/10.0	145

[a] See *Footnote a*, Table II.

Acetylcephalotaxine (VII)—A solution of 1.0 g. of cephalotaxine in 2 ml. of acetic anhydride–pyridine (1:1) was allowed to stand at room temperature for 18 hr. The resulting solution was evaporated to dryness, and the remaining oil was chromatographed on a column of Brockmann grade III neutral alumina. This procedure gave 0.9 g. of acetylcephalotaxine, m.p. 144–145°; $[\alpha]_D$ −99° (c 0.52 in chloroform), $[\alpha]_D$ −133° (c 0.05 in ethanol); λ_{max}: 290 (log ϵ 3.63), λ_{min}: 261 nm. (log ϵ 2.69); ν_{max}: 1734 cm.$^{-1}$. The mass spectrum of VII gave prominent ions at *m/e* 357 (M^+, 58%), 342 (10), 326 (19), 314 (27), 298 (100), 282 (14), 266 (31), 254 (8), 214 (10), 150 (22), 137 (11), and 115 (9). Found: M^+, *m/e* 357.164; $C_{20}H_{23}NO_5$ requires 357.158. Data from the NMR spectrum are recorded in Table I.

Anal.—Calc. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.22; H, 6.49; N, 3.86.

Transesterification Reactions—A typical transesterification reaction involved ≈100 mg. of thoroughly dried alkaloid (II–V as appropriate) and 2.5 ml. of 0.5 *M* base (sodium methoxide–methanol or sodium ethoxide–ethanol). The reactants were placed in a capped vial and allowed to stand in a dry atmosphere at room temperature for 5 hr. Aqueous 5% acetic acid (30 ml.) was then added, and the solution was extracted repeatedly with 30-ml. portions of chloroform. The chloroform extracts were washed with 5% acetic acid and 5% sodium carbonate solutions and dried over sodium sulfate; upon evaporation, they yielded the appropriate dimethyl or diethyl esters. The esters were finally purified by dissolving them in ether and passing them through a small column (1 g.) of Brockmann grade III neutral alumina. Cephalotaxine was isolated by adding base to the original aqueous acetic acid solution (to pH 10) and extracting the solution several times with chloroform. Evaporation of the dried chloroform extracts gave cephalotaxine, which was then recrystallized from ether.

Methyl 3-Carbomethoxy-3,6-dihydroxy-6-methylheptanoate (VIII) and Ethyl 3-Carboethoxy-3,6-dihydroxy-6-methylheptanoate (XII)—Alkaloid II (102 mg.) was transesterified (sodium methoxide–methanol) and yielded two products: I, 70 mg., m.p. 135–136°, $[\alpha]_D$ −183° (c 0.23 in chloroform); and VIII, 28 mg., colorless liquid, $[\alpha]_D$ −18° (c 0.47 in chloroform), ν_{max}: 3560 and 1740 cm.$^{-1}$. The NMR spectrum of VIII gave signals at δ 1.19 (s, 6H, two equivalent methyl groups), 2.80 (q, 2H, J = 16 Hz., geminal protons in an isolated methylene group), and 3.64 and 3.77 (2s, 3H each, carbomethoxyl groups). Two hydroxyl protons were observed (δ 1.95 and 3.95) and were readily exchanged with deuterium oxide. The mass spectrum of VIII showed no molecular ion but gave ions at *m/e* 231 (1%), 215 ($C_{10}H_{15}O_5$, 9), 171 ($C_9H_{15}O_3$, 35), 162 (25), 155 ($C_8H_{11}O_3$, 10), 116 (3), 99 ($C_5H_7O_2$, 14), 97 (C_6H_9O, 21), 59 (58), and 31 (100).

Anal.—Calc. for $C_{11}H_{20}O_6$ (VIII): C, 53.21; H, 8.12. Found: C, 53.10; H, 7.78.

Transesterification of II (111 mg.) with sodium ethoxide gave I (63 mg.) and the diethyl ester XII (36 mg.). The NMR spectra of VIII and XII are quite similar, except that XII exhibits two overlapping quartets at δ 4.20 and two overlapping triplets at δ 1.25 (two ethyl ester groups) rather than the methyl ester signals present in the spectrum of VIII.

Methyl 3-Carbomethoxy-2,3-dihydroxy-6-methylheptanoate (IX) and Ethyl 3-Carboethoxy-2,3-dihydroxy-6-methylheptanoate (XIII)—Alkaloid III (253 mg.) was transesterified (sodium methoxide–methanol) and yielded two products: I, 168 mg., m.p. 135–136°, $[\alpha]_D$ −188° (c 0.41 in chloroform); and IX, 71 mg., m.p. 92–93° (petroleum ether), $[\alpha]_D$ +37° (c 0.45 in chloroform), ν_{max}: 3600 and 1740 cm.$^{-1}$. The NMR spectrum of IX gave signals at δ 0.87 (d, 6H, J = 6 Hz., isopropyl group), 3.73 and 3.79 (2s, 3H each, carbomethoxyl groups), and 4.35 (s, 1H, proton on carbon bearing secondary hydroxyl). Two hydroxyl protons (exchangeable with deuterium oxide) were apparent as a broad singlet at δ 3.33 (s, 2H). The δ 4.35 signal shifts downfield to 5.44 upon acetylation of IX. The mass spectrum of IX showed no molecular ion but gave ions at *m/e* 189 (5%), 99 ($C_6H_{11}O$, 20), 90 ($C_3H_6O_3$, 100), and 81 (8).

Anal.—Calc. for $C_{11}H_{20}O_6$ (IX): C, 53.21; H, 8.12. Found: C, 53.42; H, 7.75.

Transesterification of III (116 mg.) with sodium ethoxide gave I (54 mg.) and the diethyl ester XIII (29 mg.). The NMR spectra of IX and XIII are quite similar, except that XIII exhibits two overlapping quartets at δ 4.22 and two overlapping triplets at δ 1.28 (two ethyl ester groups) rather than the methyl ester signals present in the spectrum of IX.

Methyl 3-Carbomethoxy-3,7-dihydroxy-7-methyloctanoate (X) and Ethyl 3-Carboethoxy-3,7-dihydroxy-7-methyloctanoate (XIV)—Alkaloid IV (111 mg.) was transesterified (sodium methoxide–methanol) and yielded two products: I, 68 mg., m.p. 136–137°, $[\alpha]_D$ −189° (c 0.51 in chloroform); and X, 37 mg., m.p. 34–35° (petroleum ether), $[\alpha]_D$ −18° (c 0.70 in chloroform), ν_{max}: 3560 and 1740 cm.$^{-1}$. The NMR spectrum of X gave signals at δ 1.18 (s, 6H, two equivalent methyl groups), 2.78 (q, 2H, J = 16 Hz., geminal protons in an isolated methylene group), and 3.64 and 3.77 (2s, 3H each, carbomethoxyl groups). Two hydroxyl protons were observed (δ 2.00 and 3.80) and were readily exchanged with deuterium oxide. The mass spectrum of X gave ions at *m/e* 245 (14%), 229 ($C_{11}H_{17}O_5$, 38), 185 ($C_{10}H_{17}O_3$, 84), 169 ($C_9H_{13}O_3$, 30), 167 (73), 162 (20), 145 ($C_7H_{13}O_3$, 100), 129 ($C_6H_9O_3$, 55), 116 ($C_5H_8O_3$, 34), 113 ($C_6H_9O_2$, 44), and 111 ($C_7H_{11}O$, 27). No molecular ion was observed; however, an $M^+ + 1$ ion was detected with an excessive sample pressure, *m/e* 263 (2%).

Transesterification of IV (100 mg.) with sodium ethoxide gave I (54 mg.) and the diethyl ester XIV (32 mg.). The NMR spectra of X and XIV are quite similar, except that XIV exhibits two overlapping quartets at δ 4.20 and two overlapping triplets at δ 1.25 (two ethyl ester groups) rather than the methyl ester signals present in the spectrum of X.

Anal.—Calc. for $C_{14}H_{26}O_6$ (XIV): C, 57.91; H, 9.03. Found: C, 57.91; H, 8.92.

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