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STRUCTURES OF HARRINGTONINE, ISOHARRINGTONINE, AND HOMOHARRINGTONINE

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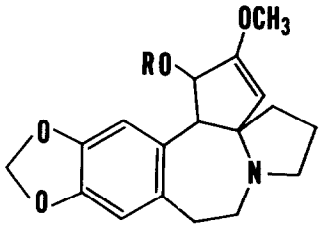
In recent communications (1,2) the structure of cephalotaxine (I) was reported and a partial structure was given for harringtonine (II). Harringtonine, as well as some companion alkaloids, shows significant inhibitory activity against experimental leukemia in mice. In the P388 leukemia system, harringtonine exhibited activity at dose levels from 0.25 to 4.0 mg./kg. and isoharringtonine (III) was active from 0.75 to 12.0 mg./kg. (3). We now report additional evidence for the structure of harringtonine (II) as well as partial structures for two other Cephalotaxus alkaloids--isoharringtonine (III) and homoharringtonine (IV).



I, R=H

II, R=$C_{10}H_{17}O_5$ } isomeric

III, R=$C_{10}H_{17}O_5$ }

IV, R=$C_{11}H_{19}O_5$

Isoharringtonine (III) was first isolated from an extract of Cephalotaxus harringtonia seed along with I, II, and a trace of IV. More recently these alkaloids have been obtained from the roots, stems, and bark of C. harringtonia. Alkaloids II, III, and IV all yield cephalotaxine (I), m.p. 136-137°C. when transesterified (sodium methoxide-methanol) along with the respective dimethyl esters V, VI, and VII. Harringtonine, $C_{28}H_{37}NO_9$ (M^+, m/e 531.246; calc. m/e 531.247) and isoharringtonine, $C_{28}H_{37}NO_9$ (M^+, m/e 531.247; calc. m/e 531.247) are isomeric in the R portion of the molecule; the R portions of homoharringtonine, $C_{29}H_{39}NO_9$ (M^+, m/e 545.255; calc. m/e 545.262) and of II are homologs.

By virtue of its higher R_f, III was separated from the other alkaloids by preparative TLC on silica. Although virtually inseparable by TLC, a 1:1 mixture of II and IV was readily

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separated by a 200-tube countercurrent distribution with a solvent system of chloroform--pH 5 McIlvaine's buffer (4). The NMR spectra of II and IV (in $CDCl_3$), although similar, are distinguishable by subtle differences in several resonances. For instance, the vinyl proton resonance at δ5.07 in the spectrum of II is shifted to δ5.05 in the spectrum of IV. The NMR spectra of II and IV (in DMSO-d_6) both show two one-proton singlets and these are easily exchanged with D_2O. Thus the presence of two tertiary hydroxyl groups is indicated in the R portion of both II and IV. Since the NMR spectrum of III (in DMSO-d_6) shows a one-proton doublet and a one-proton singlet, also exchangeable with D_2O, isoharringtonine must have one secondary and one tertiary hydroxyl group in the R portion of the molecule.

Significant features of the 100 MHz NMR spectra of dimethyl esters V, VI, and VII ($CDCl_3$), together with proposed structures for each, are as follows:

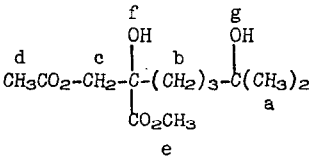
V. CH_3CO_2(e)-CH_2(d)-C(OH, g)(b)-$(CH_2)_2$-C(OH, c)$(CH_3)_2$ (a)
CO_2CH_3 (f)

	δ			δ	
a)	1.19	(6H, s)	e)	3.64 or 3.77	(3H, s)
b)	1.25-1.90	(4H, m)	f)	3.77 or 3.64	(3H, s)
c)	1.95	(1H, s)	g)	3.95	(1H, s)
d)	2.80	(2H, q, J = 16 Hz)			

VI. CH_3CO_2(f)-CH(h)(OH, e)-C(OH, d)(g)-$(CH_2)_2$(c)-CH(b)$(CH_3)_2$(a)
CO_2CH_3 (g)

	δ			δ	
a)	0.87	(6H, d, J_{CH_3H} = 6 Hz)	f)	3.73 or 3.79	(3H, s)
b,c)	1.10-2.20	(5H, m)	g)	3.79 or 3.73	(3H, s)
d,e)	3.33	(2H, s)	h)*	4.35	(1H, s)

* This signal shifts downfield to δ5.44 upon acetylation.

VII. 

	δ			δ	
a)	1.18	(6H, s)	e)	3.77 or 3.64	(3H, s)
b)	1.20-1.80	(6H, m)	f)	3.80	(1H, s)
c)	2.78	(2H, q, J = 16 Hz)	g)	2.00	(1H, s)
d)	3.64 or 3.77	(3H, s)			

s = singlet, m = multiplet, d = doublet, q = quartet.

In addition to methoxyl resonances assigned to the vinyl ether group, there are signals at δ3.55 in the NMR spectra ($CDCl_3$) of alkaloids II and IV and at δ3.58 in the spectrum of III. We assign these signals to a carbomethoxy grouping in the R portion of these compounds. Possible alternative interpretations were ruled out by transesterifying II, III, and IV with sodium ethoxide-ethanol. In addition to I, alkaloid II yielded a diethyl ester. In its NMR spectrum the two singlet resonances associated with carbomethoxy groups (e and f in V) were replaced by two A_3X_2 systems--overlapping triplets centered at δ1.22 and 1.28 with overlapping quartets at δ4.12 and 4.25. Ethanolysis of III and IV under the same conditions provided diethyl esters with spectral characteristics similar to the one derived from II.

Although molecular ions were absent in the mass spectra of esters V and VII, an $M^+ + 1$ ion was detected at m/e 263 with an excessive sample pressure of VII (5). Mass spectra demonstrated a series of homologous ions from V and VII of which the following ions were representative (formulas given for peaks determined by high resolution): from V, m/e 231, 215 ($C_{10}H_{15}O_5$), 171 ($C_9H_{15}O_3$), 155 ($C_8H_{11}O_3$), 99 ($C_5H_7O_2$), 97 (C_6H_9O); from VII, m/e 245, 229 ($C_{11}H_{17}O_5$), 185 ($C_{10}H_{17}O_3$), 169 ($C_9H_{13}O_3$), 113 ($C_6H_9O_2$), 111 ($C_7H_{11}O$). The spectrum of V contained intense peaks at m/e 162 and 130 ($C_5H_6O_4$) while VII had a 116 ($C_5H_8O_3$) peak and its 162 ($C_6H_{10}O_5$) peak was much less prominent than in V. The spectrum of VII contained intense peaks at m/e 145 ($C_7H_{13}O_3$) and 129 ($C_6H_9O_3$); however, no equivalent or homologous ions were found in the spectrum of V. This latter observation indicates some differences in the fragmentation patterns of these two compounds due to the different numbers of methylene groups in the main carbon chains.

In the mass spectrum of VI, no molecular ion was apparent and the peak of the highest mass appeared at m/e 189 ($M^+ - 59$). Other prominent peaks in the spectrum of VI were observed at m/e 99 ($C_6H_{11}O$), 90 ($C_3H_6O_3$ base peak), 81, 71, 59, and 43.

At present, it is not apparent which of the two possible carboxyl groups of V, VI, or VII was originally esterified to cephalotaxine in alkaloids II, III, and IV. However, we are continuing our investigations of these and other Cephalotaxus alkaloids and will publish more complete results later.

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