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STRUCTURE OF CEPHALOTAXINE AND RELATED ALKALOIDS

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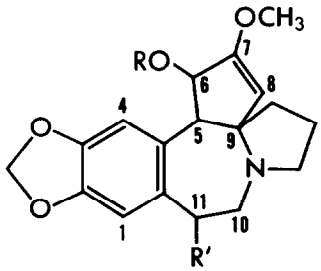
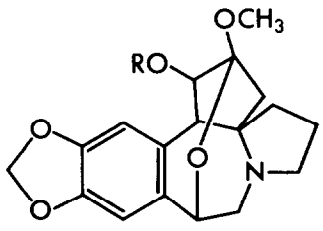
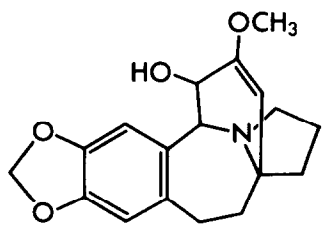
We wish to report the structure of cephalotaxine (1a), mp 132-133°, $[\alpha]_D$ -183° (c. 0.22, ethanol), and three related alkaloids (2, 3a, and 4a) isolated from Cephalotaxus harringtonia variety drupacea (1,2). These were obtained from an ethanol extract of the seed by countercurrent distribution and subsequent thin-layer chromatography of an alkaloid concentrate. An ester of cephalotaxine (2, $C_{28}H_{37}NO_9$), for which we propose the name harringtonine, has shown significant inhibitory activity against the experimental lymphoid leukemia systems L1210 and P388 in mice at 1.0 mg./kg. (3).







1a R=H, R'=H

1b R=COCH$_3$, R'=H

2 R=$C_{10}H_{17}O_5$, R'=H

3a R=H, R'=OH

3b R=COCH$_3$, R'=OCOCH$_3$

4a R=H

4b R=COCH$_3$

5

NMR evidence (Table I) favors structures 1a or 5 for cephalotaxine (M^+ m/e 315, $C_{18}H_{21}NO_4$) over other possible alternatives. The NMR spectrum contains two singlets, each equal to one proton, at δ 6.65 and 6.61. The peaks are assigned to para aromatic protons (H_1 and H_4) adjacent to oxygen functions (4). A peak with an area equivalent to two protons is

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TABLE I

Chemical shifts (δ) of Cephalotaxus harringtonia alkaloids and derivatives[a]

Compound	H_1	H_4	H_5	H_6	H_8	H_{10}	H_{11}	$O\!\!\diagdown\!\!\diagup\!\!O$ CH_2	OCH_3	$OCOCH_3$
1a	6.65	6.61	3.63	4.70	4.89	--	--	5.86	3.70	--
1b	6.59	6.57	3.77	5.80	5.05	--	--	5.85	3.71	1.58
2	6.61	6.54	3.77	5.99	5.07	--	--	5.85	3.68 3.57[b]	--
3a	6.88	6.62	3.48	4.48	4.68	3.21[c]	4.78	5.91	3.71	--
3b	6.70	6.58	3.57	5.71	4.74	3.26[c]	6.09	5.90	3.68	1.85 2.06
4a	6.65	6.65	3.45	3.99	1.49 2.65	3.05[c]	4.87	5.82	3.47	--
4b	6.63	6.51	3.75	4.78	1.55 2.70	3.07[c]	4.90	5.87	3.45	1.66

[a] Measured in $CDCl_3$ with a Varian HA-100 referenced to TMS; spin decoupling was used extensively to verify assignment.

[b] The ester contains a second methoxy group in the R portion.

[c] Center of the AB portion of an ABX system.

observed at δ 5.86 in the spectrum of 1a. This peak and an infrared band at 930-940 cm^{-1} are characteristic of protons in methylenedioxy groups attached to an aromatic ring. The spectrum contains an olefinic proton resonance as a singlet at δ 4.89 (H_8); the absence of coupling suggests that there are no vicinal protons. A low-field doublet appears at δ 4.70 (H_6), and this proton is coupled to one whose signal appears at δ 3.63 (H_5; $J_{5,6}$ = 9.2 Hz). Upon acetylation (1b) the low-field resonance moves downfield 1.10 p.p.m., a change that shows the proton is attached to the same carbon atom as a hydroxyl function (5). The position of the methoxyl resonance in 1a, at δ 3.70, indicates it is attached to an unsaturated carbon; this assignment is supported by a strong infrared absorption peak at 1650 cm^{-1}. Resonances due to para aromatic protons, as well as methylenedioxy protons, are common features of the NMR spectra of all the alkaloids studied.

The NMR data do not distinguish structure 1a from alternative 5 although assuming a phenylalanine-related precursor, 1a would be favored on biogenetic grounds. Conversion of cephalotaxine to the methiodide, mp 165-180° (decomp.), was accompanied by no skeletal

rearrangements, as shown by an NMR study with DMSO-d_6 solutions. An X-ray crystallographic study of cephalotaxine methiodide has established that 1a is the correct structure for cephalotaxine (6). In contrast to chromatographically pure (TLC) cephalotaxine, the crystalline methiodide obtained in 32% yield was optically inactive and racemic (6). Amorphous material isolated from the mother liquor was optically active however, $[\alpha]_D$ 112° (c. 0.50, ethanol), suggesting that cephalotaxine may occur as a partial racemate.

Compound 3a (M^+ m/e 331, $C_{18}H_{21}NO_5$) contains an additional hydroxyl function as evidenced by the formation of a diacetate (3b). The methine proton attached to the carbon bearing the second hydroxyl function in 3a gives rise to an apparent triplet at δ 4.78. Spin decoupling experiments revealed this multiplet to be the X portion of an ABX system and is therefore assigned to the proton on C_{11}. Since the X proton (H_{11}) in diacetate 3b moves downfield 1.31 p.p.m., it is attached to a carbon bearing a hydroxyl group. The AB resonance is centered at δ 3.21 and is assigned to the protons on C_{10}. The second hydroxyl function is placed at the benzylic position, a favored site for biogenetic oxidations.

Compound 4a (M^+ m/e 331, $C_{18}H_{21}NO_5$) can be visualized as having been formed by addition of the benzylic hydroxyl across the double bond. Dreiding models reveal close proximity of the hydroxyl to the double bond and easy formation of a relatively unstrained cage-like structure. Support for this structure is given by the absence of an olefinic absorption in the NMR spectrum. The ABX system observed in 3a is also present in 4a, but unlike 3a, acetylation does not significantly alter the chemical shift of the proton on C_{11}. Therefore, this proton is not attached to a carbon bearing a free hydroxyl group, an observation consistent with the proposed acetal structure. Structures 4a and 4b contain an isolated methylene group at C_8 which is accounted for in their NMR spectra by pairs of doublets centered at δ 2.65 and 1.49 (J = 14 Hz) for 4a and δ 2.70 and 1.55 (J = 14 Hz) for 4b. These peaks are not present in the spectra of 1, 2, or 3. The observed splitting of 14 Hz is reasonable for geminal protons.

Upon base hydrolysis, 2 [$\bar{M}^+$ m/e 531.2456 (Calc. $C_{28}H_{37}NO_9$, m/e 531.2468)] yields 1a and an unidentified acid. The biological activity may be associated either with that acid or the intact molecule 2. The mass spectrum of 2 gives m/e 298 (P - $C_{10}H_{17}O_6$) as the base peak; 1a also contains a prominent peak at m/e 298 (P - OH). The presence of a trace component was noted in the mass spectrum of 2 [$\bar{M}^+$ m/e 545.2626 ($C_{29}H_{39}NO_9$)]. We are continuing our investigations and will publish complete results elsewhere.

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