Harringtonolide, a Plant Growth Inhibitory Tropone from *Cephalotaxus harringtonia* (Forbes) K. Koch

J. George Buta,*[1a] Judith L. Flippen,[1b] and William R. Lusby[1c]

Plant Physiology Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, and Naval Research Laboratory, Washington, D.C. 20390

Received August 23, 1977

As part of a program involving a search for new naturally occurring plant regulants, we examined an ethanolic extract of the seeds of *Cephalotaxus harringtonia* (Taxaceae).[2] The growth of several species of plants was inhibited by applications of this extract. A group of tumor-inhibiting alkaloids, the harringtonines, had been isolated from this yew and characterized.[3] We tested isoharringtonine and the mixed alkaloids obtained from *C. harringtonia* in several plant bioassays but found no significant activity. Work was then begun to isolate and characterize the principal plant growth inhibitor present in the *C. harringtonia* seeds, and we now report on the isolation and the physical and chemical properties of the inhibitor.

The 2-propanol extract of the *C. harringtonia* seeds was first partitioned between hexane and aqueous methanol. The methanol-soluble portion was then chromatographed on Bio-Beads S-X2 in THF. The growth inhibitor was isolated by chromatography on silica gel with chloroform–acetonitrile, followed by high-performance liquid chromatography (HPLC) using a similar system. The successive steps in the purification sequence were monitored by a bean second-internode assay.[4] The active compound, harringtonolide, was isolated as a pale yellow solid for which no satisfactory elemental analysis was obtained. An empirical formula, $C_{19}H_{18}O_4$, was determined for the molecular ion by high-resolution mass spectrometry. The base peak in the spectrum was 282 indicating a facile elimination of CO.

The ultraviolet spectrum [λ_{max} 242, 310 nm (ϵ 20 000, 7000)] suggested the presence of a tropone moiety [cf. 4-isopropyltropone (nezukone), λ_{max} 230, 310 nm (ϵ 30 000, 15 000)].[5] The infrared spectrum contained bands assignable to lactone (1758 cm^{-1}), unsaturated ketone (1624 cm^{-1}), and olefin (1560 cm^{-1}); the latter two were similar to the 1635- and 1580-cm^{-1} bands of nezukone. The NMR spectrum contained signals among which these assignments could be made: methyl at δ 0.90 (doublet), methyl at δ 2.36 (singlet), one proton at δ 1.76 (quartet), and two protons at δ 6.92 and 6.98 (singlets).

Hydrogenation of harringtonolide over Pd/C resulted in a hexahydro product as determined by low-resolution mass spectrometry. The ultraviolet spectrum contained only end absorption. The infrared contained a lactone band (1750 cm^{-1}) and a carbonyl band at 1730 cm^{-1} with a shoulder at 1700 cm^{-1}. No band assignable to the olefinic moiety was found.

To determine the structure of harringtonolide an x-ray crystallographic analysis was undertaken. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with a = 8.38 Å, b = 22.34 Å, and c = 7.68 Å. There is one molecule per asymmetric unit corresponding to a calculated crystal density of 1.43 g/cm^3. A partial structure was obtained by application of the symbolic addition procedure for noncentrosymmetric crystals.[6] The fragment was then developed into the full structure by the tangent formula refinement and expansion method.[7] Hydrogen atoms were located in a difference map and the structure was then refined by full-matrix least-squares methods to a final R factor, agreement between observed and calculated structure factors, of 0.078. The drawing in Figure 1 which was constructed with the experimentally determined atomic positions displays the results of the x-ray analysis. Full crystallographic details will be published.[8]

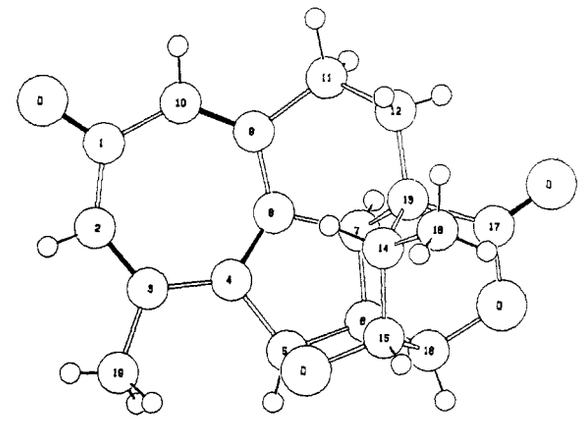

Figure 1. Molecular structure of harringtonolide as found in the crystal.

Further study of the NMR spectrum after the structure determination by x-ray crystallography permitted other assignments to be made (Table I). Confirmation of the assignment of the methyl doublet on C-18 and the adjacent proton quartet on C-14 was made by a double resonance experiment. A similar experiment indicated that irradiation of the δ 4.0 proton diminished the multiplicity of the δ 5.32 signal so the C-15 proton was assigned to δ 5.32 and C-16 to δ 4.0. The multiplets at δ 1.25 and 2.70 could be assigned to methine protons on C-6 and C-7 based on a double resonance experiment; however, only a slight collapse of the complex multiplets was obtained on irradiation of either signal. The δ 2.70 multiplet appeared to be composed of the overlap of C-12 methylene signals with those of the C-7 proton. The δ 6.92 and 6.98 signals were assigned to the protons at C-2 and C-10, consistent with data for protons α to the tropone carbonyl.[9] The ^{13}C NMR spectra of harringtonolide were obtained and assignments for the various positions were made with the aid of data obtained from an off-resonance decoupling experiment and similarities to model compounds (Table I).[10,11] The δ 186.393 for C-1 ($CDCl_3$) was near the value reported for the same carbon in tropone, δ 187.5 (CCl_4).[12]

Harringtonolide was found to be an inhibitor of plant

Table I. NMR Spectra of Harringtonolide

Carbon	^1H	^{13}C
1		186.393[a] s
2	6.92[b] s (1 H)	139.143[c] d
3		143.582[d] s
4		145.015[d] s
5	5.47 m (1 H)	79.951[e] d
6	1.25 m (1 H)	41.733 d
7	2.70 m (1 H)	49.881 d
8		145.645[d] s
9		145.855[d] s
10	6.98[b] s (1 H)	141.494[c] d
11	3.51 m (2 H)	32.281 t
12	2.70 m (2 H)	22.326 t
13		43.746 s
14	1.75 q (1 H)	39.951 d
15	5.32 m (1 H)	79.946[e] d
16	4.0 m (1 H)	85.492 d
17		173.456 s
18	0.90 d (3 H)	14.704 q
19	2.36 s (3 H)	23.839 q

[a] Multiplicity: d, doublet; m, multiplet; s, singlet; t, triplet; q, quartet. Multiplicity in ^{13}C spectra obtained through off-resonance decoupling. Values are in δ units relative to Me_4Si. [b–e] Assignments with the same superscript may be interchanged.

growth on two test species, tobacco and beans. The emulsified compound (10^{-3} M) when applied to the axils of decapitated tobacco plants effected a complete inhibition of bud growth for at least 14 days.[13] Necrosis of the meristematic tissue usually occurred and was also observed in the application of the tropone lactone to the second internode of 7-day-old bean plants. Concentrations of 1, 5, or 10 μg of the compound suspended in lanolin were sufficient to cause necrosis above the point of application within 4 days. Inhibition of growth (46%) with no indication of necrosis was observed with an application of 0.1 μg of harringtonolide to the second internode. No translocation of the harringtonolide below the point of application was seen.

Very few tropones have been found in higher plants, although the number of tropolones (2-hydroxytropones) identified in the Cupressaceae and Liliaceae is somewhat greater.[14] The latter compounds, derived from terpenes, are thought to function as fungicidal compounds in the heartwood of a number of species of trees.[15] Many terpenic lactones have been isolated from higher plants and exhibit growth regulatory activity.[16] Harringtonolide appears to be the first complex tropone containing a lactone function to be characterized. No effort has been made thus far to determine the portion(s) of the molecule responsible for the observed biological activity. We do not know whether similar compounds remain to be discovered in other *Cephalotaxus* species.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and were uncorrected. UV spectra were obtained with a Beckman 25 spectrophotometer. IR spectra were taken as KBr pellets on a Perkin-Elmer 621 spectrophotometer. ^{1}H NMR spectra were obtained at 100.1 MHz and the ^{13}C spectra at 25.2 MHz with a Varian XL-100 spectrometer. $CDCl_3$ was the solvent with Me_4Si as the internal standard. HPLC was performed on a Spectra-Physics 3500B instrument equipped with a Schoeffel 700 spectrophotometric detector. Low-resolution mass spectra were obtained with a Du Pont 21-491B spectrometer using the direct-probe method with a 70-eV ionizing voltage. High-resolution mass spectral analyses were made on an AEI MS-9 mass spectrometer by the direct-probe method using an electron-impact ionization at 70 eV. The ion source temperature was 180 °C and perfluorkerosene was the internal standard.

Isolation of Harringtonolide. Seeds of *Cephalotaxus harringtonia*[17] (2.5 kg) were ground and extracted exhaustively with *i*-PrOH at 80 °C. The resulting extract was partitioned between hexane–MeOH–H_2O (10:9:1). The MeOH-soluble portion was partioned by countercurrent distribution in four separatory funnels with the two-phase system, CCl_4–$CHCl_3$–MeOH–H_2O (280:120:320:80). The inhibitor was located in the upper phases of the four funnels by use of the bean second-internode assay. The active fraction was then applied to a gel permeation column packed with Bio-Beads S-X2 in THF. The further purified fraction was then chromatographed on a silica gel column with $CHCl_3$–CH_3CN (9:1). A R_f of 0.50 was obtained for harringtonolide on silica with $CHCl_3$–CH_3CN (4:1). The active compound was recrystallized from CH_2Cl_2 by addition of MeOH (30 mg). The final purification was done by HPLC with the detector set at 319 nm with 640 psi and a flow rate of 0.8 mL/min. The column used was 0.25 m × 4 mm with Spherisorb 5 μm silica. The solvent was $CHCl_3$–CH_3CN (9:1).

Harringtonolide. The compound was obtained as pale yellow crystals: mp 285–288 °C dec; $[\alpha]^{30}_D$ 83.0° (*c* 1.5, $CHCl_3$); UV (EtOH) λ_{max} 242 nm (ε 20 000), 310 (7000); IR (KBr) 3400, 2960, 2925, 1758, 1730 (sh), 1624, 1560, 1430, 1370, 1235, 1075, 960, 870, 750 cm^{-1}; MS *m/e* 310.1241, 310 (M^+, 21), 283 (18), 282 (M^+ − CO, 100), 225 (13), 209 (15), 207 (11), 199 (61), 197 (11), 195 (18), 181 (30), 179 (22), 169 (30), 168 (28), 167 (40), 165 (40), 153 (35), 144 (40), 143 (67), 142 (30).

Reduction of Harringtonolide. Compound (4 mg) was dissolved in EtOAc and then reduced at 45 psi of H_2 over 5% Pd/C: low-resolution MS 316 (M^+, 89), 314 (71), 312 (32), 298 (36), 282 (17), 258 (74), 55 (100).

Plant Bioassays. Harringtonolide was applied to plants in a lanolin carrier or as an emulsified suspension prepared by dissolving the compound in THF and adding Tween 20 surfactant to give a final concentration of 1% solvent and surfactant on addition of H_2O. Xanthi tobacco was used in the assay. Beans (*Phaseolus vulgaris* cv. Pinto) were used for the second internode assay. Treatments were replicated at least twice.

Acknowledgments. The authors thank D. W. Spaulding for conducting the bean second internode assay and M. S. Greenbaum for technical assistance. The NMR analyses were performed by M. O. Mattingly of the Department of Chemistry, University of Maryland. The high-resolution analysis was performed at the Mass Spectrometry Laboratory of the Florida State University. The samples of isoharringtonine and mixed alkaloids from *Cephalotaxus* were furnished by R. G. Powell, Horticultural and Special Crops Laboratory, Northern Regional Research Center, ARS, Peoria, Ill.

Registry No.—Harringtonolide, 64761-48-4; hexahydroharringtonolide, 64761-49-5.

References and Notes

(1) (a) Plant Physiology Institute, ARS; (b) Naval Research Laboratory; (c) Agricultural Environmental Quality Institute, ARS.
(2) Some authorities place *Cephalotaxus* in a separate plant family, *Cephalotaxaceae:* A. S. Barclay and R. E. Perdue, *Cancer Treatment Rep.*, **60,** 1081 (1976).
(3) R. G. Powell, D. Weisleder, C. R. Smith, Jr., and W. K. Rohwedder, *Tetrahedron Lett.*, 815 (1970).
(4) J. W. Mitchell and G. A. Livingston, Ed., "Methods of Studying Plant Hormones and Growth Regulating Substances", USDA, 1968, p 84.
(5) Y. Hirose, B. Tomita, and T. Nakasuta, *Agric. Biol. Chem.*, **32,** 249 (1968).
(6) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21,** 849 (1966).
(7) J. Karle, *Acta Crystallogr., Sect. B.*, **24,** 182 (1968).
(8) J. L. Flippen, unpublished results.
(9) D. J. Bertelli, T. G. Andrews, Jr., and P. O. Crews, *J. Am. Chem. Soc.*, **91,** 5286 (1969).
(10) E. Breitmaier and W. Voelter, "^{13}C NMR Spectroscopy", Verlag Chemie, Weinheim/Bergstr., Germany, 1974.
(11) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972.
(12) T. Machiguchi, Y. Inagaki, M. Hoshino, and Y. Kitahara, *Chem. Lett.*, 497 (1974).
(13) J. G. Buta, *J. Agric. Food Chem.*, **23,** 801 (1975).
(14) E. Zavarin, L. V. Smith, and J. G. Biocho, *Phytochemistry*, **6,** 1387 (1967).
(15) T. Yanagawa, Y. Hirose, and T. Nakatsuka, *Mokuzai Gakkaishi*, **18,** 251 (1972).
(16) D. Gross, *Phytochemistry*, **14,** 2105 (1975).
(17) The plant material was obtained from Dr. R. E. Perdue, Jr., Medicinal Plant Resources Laboratory, BARC, Beltsville, Md. Initial samples were collected as part of the USDA program developed with the Cancer Chemotherapy National Service Center, NIH.
(18) Figure 1 was drawn using the computer program ORTEP: J. K. Johnson, "Report ORNL-3794", Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
(19) Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

A Correction on the Reduction of Dihydrocodeinone with Formamidinesulfinic Acid. Stereoselective Reduction of Dihydropseudocodeinone

Nithiananda Chatterjie,* Jason G. Umans,[1a] Charles E. Inturrisi,[1a] Wen-Tsen C. Chen,[1b] Donald D. Clarke,[1b] Surendra P. Bhatnagar,[1c] and Ulrich Weiss[1c]

Department of Neurochemistry, New York State Institute for Basic Research in Mental Retardation, Staten Island, New York 10314

Received June 29, 1977

We have shown in earlier papers[2,3] that formamidinesulfinic acid (FSA, aminoiminomethanesulfinic acid) reduces the carbonyl group of a number of 6-ketones of the morphine series with complete stereoselectivity to the corresponding secondary alcohols with β configuration of the hydroxyl. This stereoselectivity stands in marked contrast to the one observed on hydride reduction, where such ketones tend to

0022-3263/78/1943-1003$01.00/0