

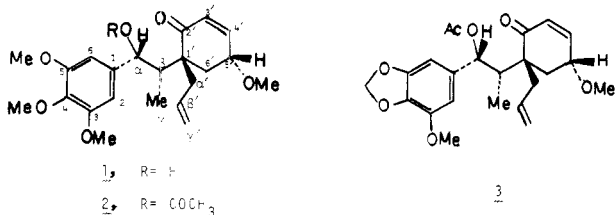
New Cytotoxic Neolignans from *Aniba megaphylla* Mez.^{1,2}S. Morris Kupchan,³ Kenneth L. Stevens,*⁴ Eric A. Rohlfing, Barry R. Sickles, Albert T. Sneden,*
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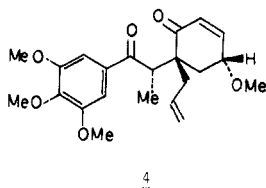
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The isolation and elucidation of the structure and stereochemistry of megaphone (1), a new cytotoxic neolignan from *Aniba megaphylla* Mez., are reported. Chemical and spectral evidence supported structure 1 for megaphone, and a direct x-ray crystallographic analysis confirmed the structure and established the stereochemistry. Two additional new cytotoxic neolignans, megaphone acetate (2) and megaphyllone acetate (3), were isolated and their structures deduced in light of the structure of 1. The lack of activity of these neolignans as inhibitors of mitosis in sea urchin eggs is discussed in terms of structural features.

An alcoholic extract of *Aniba megaphylla* Mez. (Lauraceae)⁵ was found to demonstrate inhibitory activity in vitro against cells derived from human carcinoma of the nasopharynx (KB).⁶ Systematic fractionation of the active ethanol extract (Chart I) guided by KB activity led to the isolation of three new cytotoxic neolignans, megaphone (1), megaphone acetate (2), and megaphyllone acetate (3).



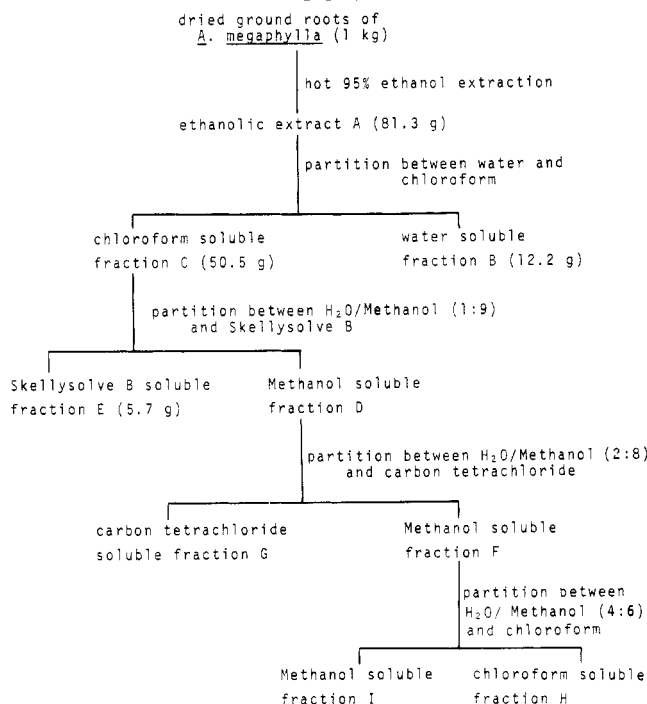
Chromatography of fraction H over silica gel gave a fraction which crystallized from chloroform-ether to afford megaphone (1). On the basis of high-resolution mass spectrometry and confirmation by elemental analysis, megaphone was assigned the molecular formula C₂₂H₃₀O₆. The NMR spectrum exhibited signals at δ 3.88 (6 H), 3.83 (3 H), and 3.46 (3 H), representing three aromatic and one aliphatic methoxy groups and suggesting that megaphone was either a lignan or neolignan. The NMR spectrum also showed a singlet at δ 6.66 (2 H) and a doublet at δ 4.64 (1 H) which, when considered with the hydroxyl stretching band at 3600 cm⁻¹ in the infrared (IR) spectrum, indicated a benzyl alcohol moiety with the aromatic ring symmetrically substituted. In order to confirm this partial structure, megaphone was oxidized using Jones reagent to give dione 4, C₂₂H₂₈O₆. The two-proton singlet was



shifted downfield in the NMR spectrum of 4, and, in the IR spectrum, the hydroxyl band disappeared and a new carbonyl band appeared at 1590 cm⁻¹, thus confirming the presence of an aryl ketone.

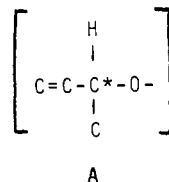
The IR spectrum of 1 showed the presence of an α,β -unsaturated carbonyl moiety (1670 cm⁻¹), and the NMR spectrum exhibited a doublet at δ 7.00 (1 H, $J = 10.5$ Hz) and a doublet of doublets centered at δ 6.02 (1 H, $J = 10.5, 2.2$ Hz) which could be assigned to the α and β protons, respectively, in a *cis*- α,β -unsaturated carbonyl system. Oxidation of 1 to afford 4 gave little change in either the IR or the NMR signals assigned to this moiety, indicating that the α,β -unsaturated carbonyl portion of the molecule was unaffected.

Further inspection of the NMR spectrum of 1 revealed the presence of an allyl group [δ 5.83 (1 H, m), 5.30 (1 H, br), 5.19

Chart I. Fractionation of the Cytotoxic Extract from *Aniba megaphylla* Mez.

(1 H, br)] and a secondary methyl group [δ 0.77 (3 H, d, $J = 7.1$ Hz)]. In the NMR spectrum of 4, the doublet for the secondary methyl group shifted to δ 1.18, indicating that it was in close proximity to the benzylic alcohol. Additionally, in the NMR spectrum of 4, the signal for the proton on the carbon α to the secondary methyl group appeared as a quartet at δ 4.04 (1 H, $J = 7.5$ Hz), indicating that this carbon was attached to two quaternary carbons.

The data thus accounted for all but one site of unsaturation, suggesting that there was an additional ring which included the α,β -unsaturated ketone. The ¹³C NMR spectrum of megaphone confirmed the presence of all the above moieties, and the presence of six quaternary carbons was indicated—four in the aromatic moiety, one in the carbonyl group, and one in the cyclohexenone ring. Consequently, the cyclohexenone ring must also contain one methylene carbon. In addition, signals were observed for two carbons with general structure A. One signal was ascribed to the benzylic carbon



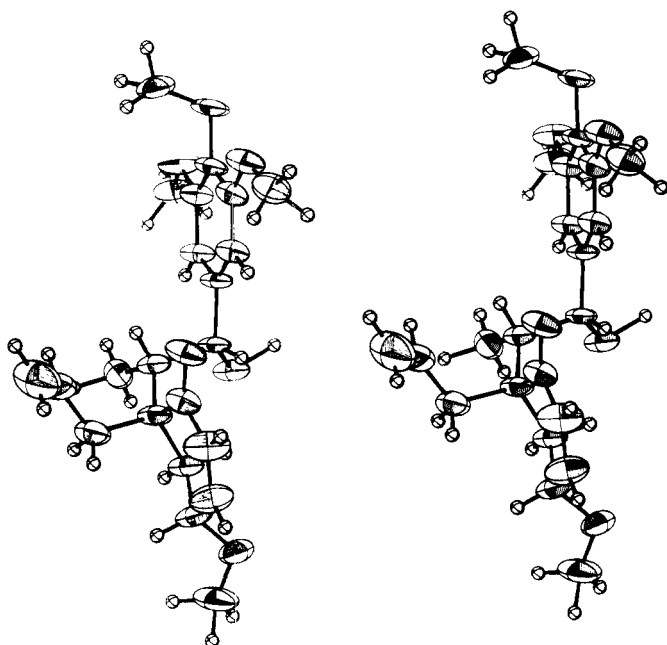
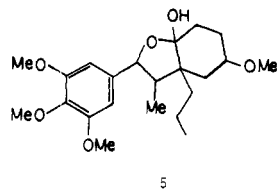


Figure 1. Stereoscopic view (ORTEP) of the molecular conformation.

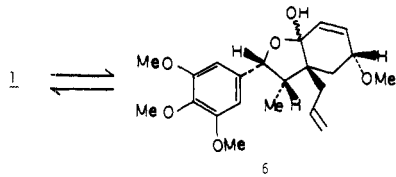
and the other to the carbon in the cyclohexenone ring attached to the methoxyl group.

Oxidized megaphone (4) showed a one-proton doublet of doublets at δ 2.90 ($J = 10.5, 12.5$ Hz) which could be assigned to one of the methylene protons in the cyclohexenone ring. Irradiation of this signal partially collapsed the multiplet at δ 4.30 (MeO-CH), and irradiation of the multiplet at δ 4.30 partially collapsed the doublet of doublets, thus confirming that the methylene group was in close proximity to the MeO-CH moiety.

Hydrogenation of megaphone (1) in EtOH gave an oil which analyzed for $C_{22}H_{34}O_6$ (by high-resolution mass spectrometry) and showed hydroxyl bands in the IR spectrum [3600 (s) and 3425 cm^{-1} (br)] but no carbonyl group. Attempts to oxidize the product with Jones reagent were unsuccessful, suggesting that the secondary alcohol was no longer present. Therefore, a hemiketal structure, 5, was proposed for the product of the hydrogenation reaction. The NMR data were also consistent with such a structure.



Careful reexamination of the NMR spectrum of megaphone (1) revealed "spurious" signals, e.g., δ 0.60 (d, $J = 7.5$ Hz), 3.37 (s), and 6.48 (s). These signals could be accounted for by the presence of an equilibrium mixture of megaphone and its hemiketal, 6, in solution. The relative intensity of the signals ascribed to 1 and 6 were found to vary with both solvent and



temperature. In $CDCl_3$ at $23^\circ C$ the ratio of 1 to 6 was found to be 100:12, whereas in pyridine- d_5 at $23^\circ C$ the ratio was

Table I. Atomic Parameters for the Nonhydrogen Atoms^a

Atom	x/a	y/b	z/c	B
C(1)	7706 (8)	1283 (7)	5130 (8)	3.4
C(2)	8802 (9)	819 (8)	4937 (8)	3.8
C(3)	10168 (8)	1067 (7)	5887 (8)	3.5
C(4)	10105 (9)	1798 (7)	6915 (8)	3.7
C(5)	8684 (10)	2225 (7)	7130 (8)	3.9
C(6)	7338 (9)	1993 (8)	6191 (8)	3.7
C(7)	11672 (10)	-229 (10)	4940 (10)	5.6
C(8)	12248 (12)	2991 (9)	7447 (11)	6.6
C(9)	7345 (11)	3269 (9)	8473 (9)	5.2
C(α)	5897 (8)	1123 (7)	4084 (8)	3.4
C(β)	5845 (8)	1956 (7)	2904 (8)	3.5
C(γ)	6608 (10)	1472 (9)	1828 (9)	4.4
C(α')	4182 (10)	3272 (8)	1200 (9)	4.6
C(β')	5298 (12)	4225 (9)	1576 (10)	6.0
C(γ')	5062 (17)	5225 (11)	1693 (13)	9.9
C(1')	4154 (9)	2398 (7)	2366 (8)	3.8
C(2')	3527 (10)	3028 (7)	3425 (8)	3.6
C(3')	1879 (11)	3144 (9)	3297 (10)	5.7
C(4')	867 (10)	2563 (10)	2373 (10)	5.4
C(5')	1306 (9)	1797 (8)	1429 (9)	4.5
C(6')	3025 (9)	1425 (7)	1830 (8)	3.8
C(7')	-179 (14)	389 (9)	54 (12)	6.8
O(3)	11579 (6)	664 (6)	5804 (6)	5.0
O(4)	11450 (7)	2060 (6)	7873 (6)	5.0
O(5)	8770 (7)	2930 (6)	8184 (6)	4.8
O(α)	5772 (6)	0000 (-) ^b	3566 (6)	4.2
O(2')	4429 (7)	3475 (6)	4384 (5)	4.7
O(5')	358 (7)	815 (6)	1359 (6)	5.3

^a Positional parameters for the hydrogen atoms (see supplementary material) are given as fractions of the unit-cell edges ($\times 10^4$) with esd's, in parentheses, on the same scale. Equivalent anisotropic thermal parameters (see supplementary material) are given in \AA^2 . ^b Held fixed to define the origin.

85:100. The temperature dependence was shown by the ratio in pyridine- d_5 at 46 and at $83^\circ C$ which was 100:95 and 100:54, respectively.

In both megaphone hemiketal (6) and tetrahydromegaphone hemiketal (5), the methyl group occurs at high field in the NMR spectrum, i.e., δ 0.60 ($J = 7.5$ Hz) and 0.62 ($J = 7.6$ Hz), respectively, thus indicating a cis relationship to the aromatic system, as found in similar neolignans from other *Aniba* species such as porosin.^{7,8} The coupling constant of the benzylic proton (δ 5.25, $J = 9.7$ Hz) in 5 indicated a gauche arrangement to the adjacent proton. Examination of the double doublet at δ 2.90 (one proton of the ring methylene group) showed coupling constants of 12.7 and 10.5 Hz, one of which was the geminal coupling constant. The other, because of its magnitude, must be the result of a 1,2-trans diaxial interaction; hence, the aliphatic methoxyl group was in an equatorial position.

From these data the structure of megaphone could be assigned as 1. To confirm this structure, and to establish the molecular conformation and absolute stereochemistry, a direct x-ray analysis of 1 was carried out. A stereoscopic view⁹ of the molecular structure found is shown in Figure 1, and atomic coordinates are given in Table I. Bond distances and angles and selected torsion angles are given in Figure 2, and additional torsion angles of interest are listed in Table II.

The molecular structure proposed is confirmed as correct. In the crystal the molecule adopts an extended stepped conformation with the bond C(α)-C(β) lying in a plane near normal to the plane of the phenyl ring, the torsion angle C(1)-C(α)-C(β)-C(1') being -143° . The more favorable, fully staggered, 180° alignment is prevented by the limiting intramolecular contact O(α)-H(6'a) of 2.54 \AA which prevents further rotation about C(α)-C(β). The hydroxy group O(α)

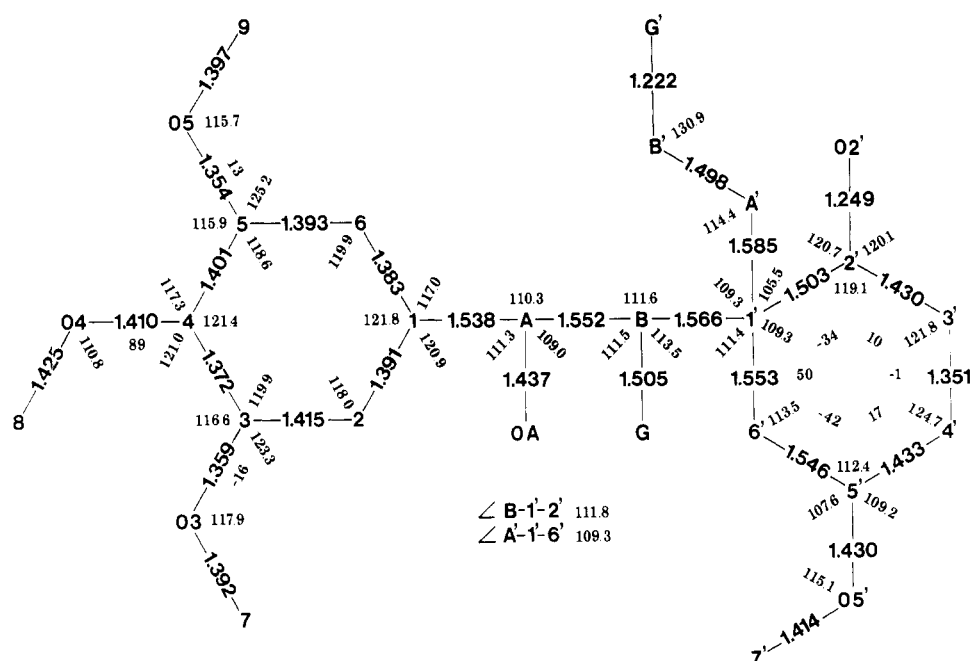


Figure 2. Bond lengths (Å), bond angles (degrees), and selected torsion angles (degrees) in the molecule of megaphone. Estimated standard deviations in bond distances are in the range 0.010–0.016 Å, in bond angles from 0.7 to 1.1°, and in torsion angles $\sim 2^\circ$.

Table II. Selected Exocyclic Torsion Angles (Degrees)

C(6)–C(1)–C(α)–C(β)	94	C(α)–C(β)–C(1')–C(α')	178
C(6)–C(1)–C(α)–O(α)	–145	C(α)–C(β)–C(1')–C(2')	62
C(2)–C(1)–C(α)–C(β)	–80	C(α)–C(β)–C(1')–C(6')	–61
C(2)–C(1)–C(α)–O(α)	41	C(γ)–C(β)–C(1')–C(γ')	–55
C(1)–C(α)–C(β)–C(γ)	89	C(γ)–C(β)–C(1')–C(2')	–171
C(1)–C(α)–C(β)–C(1')	–143	C(γ)–C(β)–C(1')–C(6')	66
C(β)–C(1')–C(α')–C(β')	–55	C(1')–C(α')–C(β')–C(γ')	–113

is *cis* to the C(γ) methyl group, one of whose protons, H(γ_b), comes within 2.25 Å of H(α'_b) of the allyl side chain to dictate the slightly less than optimal value of -55° for the torsion angle C(β)–C(1')–C(α')–C(β').

The cyclohexenone ring does not adopt the 1,2-diplanar conformation¹⁰ which would lead to coplanarity of the five atoms, O(2'), C(2'), C(3'), C(4'), and C(5'), but instead adopts a flattened monoplanar (half-chair) conformation defined by the parameters $\Delta C_2(3'-4')$ 7.5° , $\Delta C_s(3')$ 13.1° , and $\Delta C_s(4')$ 22.6° .¹¹ The four atoms C(2'), C(3'), C(4'), and C(5') are rigorously coplanar, with the maximum deviation of an atom from their least-squares mean plane¹² being only 0.003 Å. C(6') is displaced to one side of that plane by 0.41 Å and C(1') to the other side by 0.22 Å. The 10° endocyclic torsion angle about C(2')–C(3') may be traced to the limiting contact O(2') \cdots H(β) of 2.52 Å. A similar half-chair conformation [$\Delta C_2(2-3)$ 3.6° , $\Delta C_s(3)$ 15° , and $\Delta C_s(1)$ 40.9°] has also been noted in the similarly substituted A ring of 4 α -bromo-5 α -androst-2-ene-1,17-dione.¹³

Bond lengths and valence angles in the molecule show expected deviations from ideal values and regular geometry. Steric crowding at C(1') is reflected in the longer than usual C_{sp^3} – C_{sp^3} bond distances involving that atom, and the steric interactions between the in-plane methyl groups C(7) and C(9) and the ring protons lead to a familiar asymmetry of exocyclic valence angles at C(3) and C(5).¹⁴ The two C–O–CH₃ bond angles for these groups are also significantly larger than that at O(4), again for the same steric reason, and this is also reflected in the differing C–O bond lengths in the three groups, the significant lengthening of C(4)–O(4) by comparison with C(3)–O(3) and C(5)–O(5) being attributable to loss of orbital overlap between O(4) and the phenyl ring. The terminal

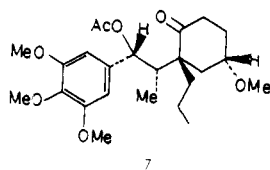
CH=CH₂ bond distance in the allylic moiety is much shorter than a normal ethylenic double bond, and the conformation adopted about C(α')–C(β'), with $\phi = -113^\circ$, has H(γ'_b) and H(α'_a) eclipsed and only 2.45 Å apart, reflected in the larger than usual valence angle at C(β').

In the trimethoxyphenyl moiety, the two flanking –OCH₃ groups lie approximately in the plane of the phenyl ring, whereas the central –OCH₃ group lies in a plane inclined nearly normal to the ring. The three exocyclic C–O torsion angles are, in sequence from C(3) to C(5), -16 , 89 , and 13° . This pattern of torsion angles is similar to that observed in reserpine¹⁵ and in mescaline hydrobromide¹⁶ but differs from that characteristic of a variety of trimethoxyphenyl compounds showing activity as inhibitors of mitotic spindle formation.¹⁷ These include certain colchicine derivatives,¹⁸ steganacin and steganangin,¹⁹ and podophyllotoxin²⁰ whose structures have been determined crystallographically. In these inhibitors, steric factors lead to a significantly nonzero exocyclic C–O torsion angle for one of the flanking –OCH₃ groups, as well as for the central group, with a value near $\pm 90^\circ$ being associated with greater inhibitory activity^{18b} in colchicine derivatives. Margulis^{18b} has suggested that the pattern of methoxy group orientation provides specificity in recognition of colchicine derivatives by a component of the microtubule structure by regulating the accessibility of a portion of the benzenoid ring. Both *cis* and *trans* arrangements of the two out-of-plane methoxy groups have been observed in inhibitors, and it therefore seems likely that if the potential acceptor of the flanking –OCH₃ site is a hydrogen bond donor it will be coplanar with the phenyl ring and, one may speculate, most probably lies along the direction which would be occupied by an in-plane O–CH₃ bond. This neolignan lacks the 90,90,0° pattern proposed as a requirement, and tests show²¹ that neither it nor 2 shows measurable activity as inhibitors of spindle formation in sea urchin eggs.

In the crystal the molecules are linked in chains along the twofold screw axis by hydrogen bond formation between the O(α) hydroxy group of one molecule and the O(2') carbonyl oxygen of an immediate neighbor (O \cdots O, 2.80 Å). Other intermolecular contacts correspond to normal van der Waals separations.

Chromatography of fraction G over silica gel gave two active

(KB) fractions. The more polar fraction was subjected to extensive preparative TLC leading to megaphone acetate (2), $C_{24}H_{32}O_7$. Acetylation of megaphone (1) also afforded acetate 2 which was identical with the natural material. Hydrogenation of 2 gave a tetrahydro derivative, 7. Inspection of the IR



and NMR spectra of both 2 and 7 indicates that there is no hemiketal formation in either case, thus confirming structure 2 for megaphone acetate.

The less polar active fraction from the chromatography of fraction G gave, after further chromatography, megaphyllone acetate (3), $C_{23}H_{28}O_7$. 3 differs from megaphone acetate (2) only in the replacement of two adjacent methoxyl groups in the aromatic ring with a methylenedioxy moiety. Structure assignment for 3 was based primarily on comparison of its NMR spectrum with that of 2.

Experimental Section

General. Melting points were determined on a Mettler Model FP2 hot stage and are uncorrected. Ultraviolet absorption spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Model 337 recording spectrophotometer. Nuclear magnetic resonance spectra were determined on a JEOL PS-100 pulsed FT NMR spectrometer interfaced to a Texas Instruments JEOL 980A computer, with tetramethylsilane as an internal standard. Mass spectra were determined on Hitachi Perkin-Elmer Model RMU-6E and AEI Model MS-902 spectrometers. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. All thin-layer chromatography was carried out on silica gel 60 precoated plates, F-254 (E. Merck). Visualization of TLC was effected with short-wave UV and concentrated sulfuric acid–vanillin–ethanol (20:1:3) spray.

Extraction and Preliminary Fractionation. The ground root (1 kg) of *A. megaphylla* was continuously extracted with hot 95% ethanol for 24 h and the ethanol extract concentrated under reduced pressure to a dark brown residue (A, 81.3 g). Fraction A was partitioned between water (2 L) and chloroform (1 L) to give fractions B (12.2 g) and C (50.5 g), respectively. Fraction C was further partitioned between methanol–water (9:1, 0.5 L) and Skellysolve B (0.5 L) to give fractions D and E (5.7 g), respectively. Further partitioning of D with methanol–water (8:2, 1 L) and carbon tetrachloride (300 mL) gave fractions F and G (24.3 g), respectively. Final partitioning of F was carried out with methanol–water (6:4, 1 L) and chloroform (300 mL) to give fraction I (0.4 g) and H (17.4 g), respectively.

Megaphone (1). Fraction H was chromatographed on silica gel 40 (10% water, 1 kg) eluting with ether. Megaphone was crystallized from chloroform–ether: mp 151.5–152.5 °C (273 mg, 0.027%); $[\alpha]_D^{27} -23^\circ$ (c 0.15, EtOH); UV λ_{max} (EtOH) 279 nm sh (ϵ 334), 269 (501); IR (CCl₄) 3600, 3375, 2938, 2830, 1670, 1590, 1505, 1460, 1420, 1330, 1235, 1130, 1012 cm⁻¹; NMR (CDCl₃) δ 7.00 (1 H, d, $J = 10.5$ Hz, $-\text{CH}=\text{C}$), 6.66 (2 H, s, aromatic), 6.48 (s, aromatic hemiketal), 6.02 (1 H, dd, $J = 10.3, 2.2$ Hz, $-\text{CH}=\text{C}$), 5.83 (1 H, m, $-\text{CH}=\text{C}-$), 5.30 (1 H, br, $\text{CH}_2=\text{C}$), 5.19 (1 H, br, $\text{CH}_2=\text{C}$), 5.02 (1 H, s, $-\text{OH}$), 4.64 (1 H, d, $J = 1.5$ Hz, Ar-CH-OH), 4.23 (1 H, m, MeO-CH-), 3.88 (6 H, s, MeO-), 3.83 (3 H, s, MeO-), 3.46 (3 H, s, MeO-), 3.37 (s, MeO, hemiketal-OMe), 2.57–2.19 (4 H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$ and MeO-C'H-CH₂), 1.96 (1 H, br q, $J = 6.9$ Hz, CH_3CH), 0.77 (3 H, d, $J = 7.1$ Hz, Me-), 0.60 (d, $J = 7.5$ Hz, hemiketal-Me); ¹³C NMR (CDCl₃) δ 11.84 (1 C, q, γ -C), 6.21 (hemiketal, q, γ -C), 36.3 (1 C, t, α' -C), 37.7 (1 C, t, β' -C), 44.8 (1 C, d, β -C), 52.27 (1 C, s, 1'-C), 55.82 (2 C, q, 7,9-C), 56.40 (1 C, q, 8-C), 60.53 (1 C, q, 7'-C), 71.01 (1 C, d, 5'-C), 73.15 (1 C, d, α -C), 102.46 (2 C, d, 2,6-C), 119.11 (1 C, t, γ' -C), 128.67 (1 C, d, β' -C), 132.02 (1 C, d, 3'-C), 135.13 (1 C, s, 1-C), 140.52 (1 C, s, 4-C), 149.94 (1 C, d, 4'-C), 152.51 (2 C, s, 3,5-C), 193.37 (1 C, s, 2'-C); mass spectrum (chemical ionization, methane gas) m/e 391.2117 ($M^+ + H$, calcd for $C_{22}H_{31}O_6$, 391.2120), 373.2008 (calcd for $C_{22}H_{29}O_5$, 373.2015). Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.47. Found: C, 67.66; H, 7.72.

Oxidized Megaphone (4). Megaphone (20 mg) was dissolved in 2 mL of acetone and Jones reagent added dropwise with stirring until

the color remained. Water was added and the product was extracted with ether. The ethereal extract was washed with water, dried over anhydrous magnesium sulfate, and then evaporated. The material was subjected to preparative TLC on silica gel eluting twice with ether (R_f 0.55) to give the product as an oil: $[\alpha]_D^{28} -32.8^\circ$ (c 0.21, CHCl₃); UV λ_{max} (EtOH) 280 nm (ϵ 8989); IR (CCl₄) 2940, 1677, 1580, 1505, 1465, 1418, 1322, 1132 cm⁻¹; NMR (CDCl₃) δ 7.18 (2 H, s, aromatic), 6.93 (1 H, d, $J = 10.2$ Hz, $-\text{CH}=\text{C}$), 5.96 (1 H, dd, $J = 10.0, 2.2$ Hz, $\text{CH}=\text{C}$), 5.70 (1 H, m, $-\text{CH}=\text{CH}_2$), 5.22 (1 H, s, $\text{CH}_2=\text{C}$), 5.095 (1 H, s, $\text{CH}=\text{C}$), 4.30 (1 H, m, MeO-CH), 4.04 (1 H, q, $J = 7.5$ Hz, CH_3CH), 3.90 (9 H, s, MeO-), 3.46 (3 H, s, MeO-), 2.90 (1 H, dd, $J = 10.5, 12.5$ Hz, $-\text{CCH}_2\text{COMe}$), 2.51–2.11 (3 H, m, $-\text{CH}_2\text{CH}=\text{CH}_2$ and CCH_2COMe), 1.18 (3 H, d, $J = 7.6$ Hz, $-\text{Me}$); mass spectrum m/e 388 (M^+), 224, 195.

Tetrahydromegaphone (5). Megaphone (5 mg) was dissolved in 3 mL of absolute alcohol and 10 mg of 10% Pd/C added. The mixture was stirred under an atmosphere of hydrogen for 0.5 h and then filtered to give, after evaporation of solvent, tetrahydromegaphone (5) as an oil: $[\alpha]_D^{28} -22.3^\circ$ (c 1.48, CHCl₃); UV λ_{max} (EtOH) 270 nm (ϵ 963), 279 sh (744); IR (CCl₄) 3595, 3420, 2950, 2928, 1585, 1502, 1458, 1415, 1365, 1328, 1232, 1130, 1100, 1012, 965 cm⁻¹; NMR (CDCl₃) δ 6.57 (2 H, s, aromatic), 5.25 (1 H, d, $J = 9.7$ Hz, Ar-CHO-), 3.85 (6 H, s, MeO-), 3.83 (3 H, s, MeO-), 3.34 (3 H, s, OMe), 2.78 (1 H, dd, $J = 9.7, 7.6$ Hz, $-\text{CH}_2\text{COMe}$), 2.09–1.32 (12 H, m), 0.96 (3 H, t, $J = 7$ Hz, Me-), 0.62 (3 H, d, $J = 7.6$ Hz, Me-); mass spectrum m/e 394 (M^+), 376, 198, 197, 181, 169, 155, 141, 138, 123.

Megaphone Acetate (2) (Synthetic). Megaphone (10 mg) was dissolved in 5 mL of pyridine and 0.5 mL of acetic anhydride and then heated at 50 °C for 6 h. Evaporation left a material which was submitted to preparative TLC (silica gel, eluted twice with ether) to give 10.1 mg of the acetate 2 as an oil: $[\alpha]_D^{22} -2.4^\circ$ (c 0.29, EtOH); UV λ_{max} (EtOH) 269 nm sh (ϵ 1056), 279 sh (671); IR (CCl₄) 2970, 2930, 2830, 1740, 1665, 1585, 1500, 1450, 1415, 1375, 1325, 1135, 1015, 963, 920 cm⁻¹; NMR (CDCl₃) δ 6.91 (1 H, dt, $J = 10.3, 2$ Hz, $-\text{CH}=\text{C}$), 6.55 (2 H, s, aromatic), 6.00 (1 H, dd, $J = 10.3, 2.2$ Hz, $\text{CH}=\text{C}$), 5.68 (1 H, s, Ar-CH-OAc), 5.46 (1 H, m, $-\text{CH}=\text{CH}_2$), 5.06 (1 H, s, $\text{CH}_2=\text{C}$), 4.95 (1 H, m, $\text{CH}_2=\text{C}$), 4.17 (1 H, m, CCHOMe), 3.88 (6 H, s, OMe), 3.82 (3 H, s, OMe), 3.46 (3 H, s, OMe), 2.63–1.75 (5 H, m), 2.12 (3 H, s, acetate), 0.93 (3 H, d, $J = 7.4$ Hz, $-\text{Me}$); mass spectrum m/e 432 (M^+), 266, 224, 197, 169.

Megaphone Acetate (2) (Natural). Fraction I was chromatographed on a column of silica gel 40 (5% H₂O, 1 kg) with ether saturated with water to give an active fraction (6 g) which was subjected to preparative TLC (twice with ether) on silica gel to give megaphone acetate (2) (R_f 0.52) as an oil. The material was identical in all respects with the synthetic material: mass spectrum (chemical ionization, methane gas) m/e 433.2225 ($M^+ + H$, calcd for $C_{24}H_{33}O_7$, 433.2226).

Tetrahydromegaphone Acetate (7). Megaphone acetate (2, 20 mg) was dissolved in 10 mL of absolute ethanol and stirred under a hydrogen atmosphere in the presence of 20 mg of 10% Pd/C. After 1 h the solvent and catalyst were removed leaving 17 mg of product which was purified by TLC: $[\alpha]_D^{21} -35.2^\circ$ (c 0.91, CHCl₃); UV λ_{max} (EtOH) 269 nm (ϵ 872), 278 (654); IR (CCl₄) 2950, 2930, 1738, 1700, 1585, 1503, 1455, 1418, 1325, 1230, 1130, 1104, 1012 cm⁻¹; NMR (CDCl₃) δ 6.66 (2 H, s, aromatic), 5.76 (1 H, s, Ar-CH-OAc), 3.89 (6 H, s, MeO-), 3.82 (3 H, s, MeO-), 2.12 (3 H, s, acetate), 0.85 (3 H, d, $J = 7.4$ Hz); mass spectrum m/e 436 (M^+), 376, 302, 266, 208, 197, 181, 148, 141.

Megaphyllone Acetate (3). A fraction (1.5 g) eluting just prior to megaphone acetate on chromatography of fraction I was rechromatographed on silica gel 40 (5% water, 1 kg of silica gel) with ether. The active fraction (450 mg) was again chromatographed on silica gel 40 (1 kg) with ethyl acetate–hexane (1:1) and final purification was afforded by preparative TLC on silica gel (EtOAc–hexane) to give megaphyllone acetate (3, 214 mg) as an oil: $[\alpha]_D^{22} 0^\circ$; UV λ_{max} (EtOH) 275 nm (ϵ 1248), 284 sh (1027); IR (CCl₄) 2925, 1750, 1650, 1635, 1510, 1450, 1430, 1380, 1360, 1320, 1232, 1132, 1093, 961, 920 cm⁻¹; NMR (CDCl₃) δ 6.90 (1 H, d, $J = 10.0$ Hz, $\text{CH}=\text{C}$), 6.55 (1 H, d, $J \sim 0.5$ Hz, aromatic), 6.50 (1 H, d, $J \sim 0.5$ Hz, aromatic), 5.99 (1 H, dd, $J = 10.0, 1.9$ Hz, $\text{CH}=\text{C}$), 5.93 (2 H, s, $-\text{OCH}_2\text{O}-$), 5.65 (1 H, s, Ar-CH-OAc), 5.45 (1 H, m, $-\text{CH}=\text{CH}_2$), 5.06 (1 H, s, $\text{CH}_2=\text{C}$), 4.968 (1 H, br, $\text{CH}_2=\text{C}$), 4.18 (1 H, m, $-\text{CHOMe}$), 3.93 (3 H, s, $-\text{OMe}$), 3.45 (3 H, s, $-\text{OMe}$), 2.10 (3 H, s, acetate), 2.63–1.74 (5 H, m), 0.91 (3 H, d, $J = 7.3$ Hz, $-\text{Me}$); mass spectrum (chemical ionization, methane gas) m/e 417.1928 ($M^+ + H$, calcd for $C_{23}H_{29}O_7$, 417.1913), 357.1702 (calcd for $C_{21}H_{25}O_5$, 357.1695).

Crystal Data: ²² monoclinic; space group $P2_1$; $a = 8.757$ (3), $b = 11.942$ (3), $c = 10.177$ (3) Å; $\beta = 101.29$ (1)°; $U = 1044$ Å³; $Z = 2$; D_x

= 1.242 g cm⁻³; $F(000) = 420$; Cu $K\alpha$ radiation, $\lambda = 1.5418 \text{ \AA}$, $\mu = 7.4 \text{ cm}^{-1}$.

A single crystal of megaphone (1) suitable for x-ray diffraction study was grown from a solution of chloroform-ether. Unit-cell symmetry was determined from 25° precession photographs taken with Mo $K\alpha$ radiation. The systematic absences, $0k0$ with k odd, uniquely defined the space group for this optically active material as $P2_1$. Unit-cell dimensions were found by a least-squares fit to the observed values of $\pm 2\theta$ for 20 strong general reflections measured on the diffractometer from a carefully centered crystal.

Intensity Data. A single-crystal plate $0.4 \times 0.3 \times 0.04 \text{ mm}$ was mounted with the c^* axis parallel to the ϕ axis of a Picker full-circle diffractometer controlled by an XDS Sigma 2 computer. A single quadrant of reciprocal space to $2\theta = 120^\circ$ was surveyed with Cu $K\alpha$ radiation made monochromatic by Bragg reflection from a highly oriented graphite crystal. The θ - 2θ scan method was used with a scan range of 2° and a scan speed of 2° min^{-1} . Background intensity was measured for 15 s at both the beginning and end of each scan with both crystal and counter at rest. Scintillation counting was used with pulse-height analysis. Scattered intensity significantly above background [$I > 3\sigma(I)$] was found at 1318 of the 1564 independent locations surveyed. Stability of the experimental conditions was monitored by measurement of the intensities of two reference reflections after every 50 scans. The rms deviation from the mean intensity was in each case $< 1\%$. No absorption corrections were made.

Structure Determination and Refinement. The phase problem was solved by routine application of the program MULTAN²³ using the $245E(hkl) > 1.41$. Refinement was by the block-diagonal least-squares methods (3×3 , 6×6 blocks) with anisotropic thermal parameters adopted for the nonhydrogen atoms. Hydrogen atoms, other than those of the C(7') methyl group, were located from three-dimensional difference electron-density maps and their positions optimized by the assumption of standard geometries (C-H, 1.08 Å; H-C-H 109.5°; etc.). Contributions for these atoms in fixed positions and with fixed isotropic B values were included in the least-squares calculations. The function minimized was $\sum w(|F_o| - k|F_c|)^2$, with weights assigned in a standard manner.²⁴ Convergence was assumed with the largest shift to error ratio being 0.14 and the mean ratio being 0.03. The conventional unweighted and weighted residuals were 0.076 and 0.093. Despite the high value of the latter quantity, an analysis of the distribution of weighted differences showed no obvious anomalies. A final difference electron-density map contained no structurally significant information and had no density in excess of 0.26 e/\AA^3 .

An attempt was made to establish the absolute configuration of the molecule by making use of the anomalous dispersion effect for oxygen. Separate structure-factor calculations including the $\Delta f'$ and $\Delta f''$ terms²⁵ gave unweighted R values of 0.0759 and 0.0765, the lower residual being associated with the enantiomer described. These values indicate a significant difference between the two enantiomers at the 99% confidence level by the Hamilton R -ratio test,²⁶ but we have not been able to confirm this indication by consistent measurement of significant differences between Bijvoet pairs of reflections,²⁷ and so this assignment of absolute configuration should be viewed with caution.

The scattering functions used were taken from ref 28. With the exception of ORTEP and MULTAN, for which use was made of a CDC Cyber 172 computer, all programs used were written in this laboratory for the XCS Sigma 2 computer.

Registry No.—1, 64332-37-2; 2, 64332-38-3; 3, 64332-39-4; 4, 64332-40-7; 5, 64332-41-8; 7, 64332-42-9.

Supplementary Materials Available: Atomic coordinates used for hydrogen positions, anisotropic thermal parameters for C and O atoms, equations of least-squares mean planes, and selected intramolecular and intermolecular contact distances (6 pages). Ordering information is given on any current masthead page.

References and Notes

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- (3) Deceased Oct 19, 1976.
- (4) Present address: U.S. Department of Agriculture, Albany, Calif. 94710.
- (5) We thank Dr. Robert E. Perude, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, Md., for *A. megaphylla* roots collected in March 1975 in accordance with the program developed by the National Cancer Institute.
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