wood of stems from Ethiopia also yielded active extracts. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.

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(5) *Tumor-inhibitory activity and cytotoxicity were assayed under the auspices of the National Cancer Institute, by the procedures described by* R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott [*Cancer Chemother. Rep., Part 3*, **3**, 1 (1972)].

(6) Bruceantin and bruceantinol showed potent antileukemic activity against P-388 lymphocytic leukemia, and were active over a 50- to 100-fold dosage range at the microgram per kilogram level. Bruceantarin showed moderate activity against P-388, and dehydrobruceantin, dehydrobruceantarin, isobruceine B, and the previously isolated[7] bruceine B showed only marginal activity against this system. Bruceantin, bruceantinol, bruceantarin, and isobruceine B showed cytotoxicity (ED_{50}) against KB cell culture at 10^{-2}–10^{-3} μg/ml, and dehydrobruceantin and dehydrobruceantarin at 10^{-1} μg/ml.

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Notes

Dehydroailanthinone, a New Antileukemic Quassinoid from *Pierreodendron kerstingii*[1-3]

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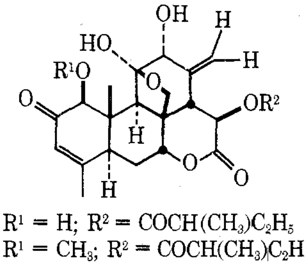
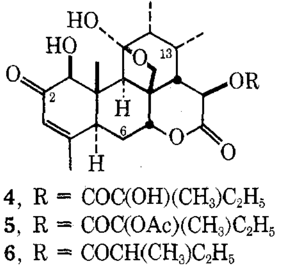
The antileukemic activity of *Brucea antidysenterica* and its active principles, bruceantin[2,4] and bruceantinol,[2] prompted us to investigate other plants of the Simaroubaceae family. An alcohol extract of *Pierreodendron kerstingii* Little[5] was found to show significant activity *in vivo* against P-388 lymphocytic leukemia in the mouse (PS) and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[6] We report herein the fractionation of an active extract of *P. kerstingii* and the isolation and structure elucidation of a new antileukemic quassinoid, dehydroailanthinone (**1**),[7] and the companion quassinoids, glaucarubinone (**4**), 2′-acetylglaucarubinone (**5**), and ailanthinone (**6**).



1, R¹ = H; R² = COCH(CH₃)C₂H₅
2, R¹ = CH₃; R² = COCH(CH₃)C₂H₅
3, R¹ = R² = H



4, R = COC(OH)(CH₃)C₂H₅
5, R = COC(OAc)(CH₃)C₂H₅
6, R = COCH(CH₃)C₂H₅
7, R = H

Fractionation of an alcohol extract, guided by assay against KB and PS, revealed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition, and the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition. Column chromatography of the aqueous methanol solubles on SilicAR CC-7 yielded KB and PS active fractions, F and G, on elution with chloroform and 2% methanol in chloroform, respectively. Rechromatography of fraction G on SilicAR CC-7 using 2% ethanol in dichloromethane gave the known glaucarubinone (**4**, 0.05%).[8]

Further fractionation of F was effected with two successive high-ratio chromatographic columns on SilicAR CC-7, first with isopropyl alcohol in dichloromethane, and then with ether in benzene as eluents, giving three major components: dehydroailanthinone (**1**), 2′-acetylglaucarubinone (**5**),[9] and ailanthinone (**6**).[9]

The molecular formula $C_{25}H_{32}O_9$ was advanced for dehydroailanthinone (**1**) on the basis of elemental analysis and mass spectral data. The presence of an α-methylbutyrate ester was indicated by the loss of 84 amu in the mass spectrum and the presence of peaks at *m/e* 85 $[O{\equiv}CCH(CH_3)CH_2CH_3]^{\cdot +}$ and 57 $[CH(CH_3)CH_2CH_3]^{\cdot +}$. Furthermore, there appeared in the nmr spectrum signals for primary and secondary methyl groups assignable to the ester and corresponding in chemical shift to the peaks assigned to the α-methylbutyrate of ailanthinone (**6**). The presence of the ring A moiety as in **1** was supported by the uv spectrum, the vinyl methyl signal in the nmr spectrum (τ 8.26), and the mass spectral fragment ions at *m/e* 247 and 151, which are common ions in quassinoids with a similar A ring and an 11,30-hemiketal in the C ring.[9]

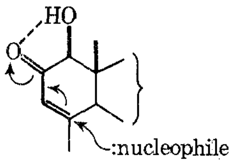
Alkaline hydrolysis of dehydroailanthinone (**1**) gave $\Delta^{13,18}$-glaucarubolone (**3**)[10] which displayed resonances in the nmr spectrum for the C-4 and C-10 methyl groups but lacked a signal corresponding to the C-13 methyl group. The presence of an AB quartet at τ 4.77 in the nmr spectrum of **1** was consistent with the presence of a 13,18-double bond. Except for these nmr spectral differences, the close similarity of all other nmr signals in the spectra of **1** and **6** strongly supported the same stereochemistry at all other positions in dehydroailanthinone (**1**) and ailanthinone (**6**).

By an isolation procedure very similar to that described, glaucarubinone (**4**) and 2′-acetylglaucarubinone (**5**) were also isolated from the wood of stems of *Picrasma excelsa* Planch., in 0.007 and 0.001% yields, respectively.

Earlier studies[11] in this laboratory have demonstrated the importance of Michael-type additions of model biological nucleophiles to highly electrophilic conjugated systems, especially methylene lactones and α,β-unsaturated esters, in relation to the cytotoxicity of several classes of terpenoids. An extension to selective alkylation of biological macromolecules has been proposed for unsaturated ketones in the cucurbitacins.[12]

Saturation of the conjugated Δ^3 double bond in the quassinoids is accompanied by a profound lessening in cytotoxicity of the resulting dihydroquassinoid derivatives.[13] It is suggested that reactions of the conjugated ketone with biological nucleophiles may play an important role in the mechanism by which quassinoids exert their biological activity. *In vitro* models for such Michael-type additions have been reported.[14]

Methylation of the C-1 alcohol of dehydroailanthinone (1) results in a diminution of cytotoxicity and of *in vivo* antileukemic activity. The decrease in activity which accompanies methylation suggests that the free hydroxyl in 1 may also play an active role in the mechanism of biological action. Possibly the hydroxyl group enhances the reactivity of the conjugated ketone toward biological nucleophiles through intramolecular hydrogen bonding, as shown. The lowered biological activity of the methyl ether 2 may result from the diminished reactivity of the conjugated ketone.



Investigations are in progress to explore further the significance of the α,β-unsaturated ketone and of other structural features in relation to the tumor-inhibitory activity of the quassinoids.

Experimental Section

General. Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Ultraviolet absorption spectra were determined on Beckman Model DK-2A and Coleman Hitachi Model EPS-3T recording spectrophotometers. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian HA-100 spectrometer or a JEOL PS-100 p FT NMR spectrometer interfaced to a Texas Instrument 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. Petroleum ether refers to the fraction with bp 60–68°. All thin-layer chromatography was carried out on prepared plates (Brinkmann, Mallinckrodt, and Camag). Visualization of tlc was effected with ceric ammonium sulfate solution.

Extraction and Preliminary Fractionation. The dried ground stem bark of *P. kerstingii* (2.1 kg)[5] was continuously extracted with hot 95% ethanol for 24 hr and the ethanol extract was concentrated under reduced pressure to a dark brown residue (A, 182 g). Fraction A was partitioned between water (1.5 l.) and ethyl acetate (1.5 l.), and the aqueous layer further extracted (2×) with ethyl acetate (1.5 l.). The aqueous layer was evaporated to give fraction B (132 g), as a brown foam. The combined ethyl acetate layers were evaporated to give C (50 g), which was then partitioned between 20% aqueous methanol (1 l.) and petroleum ether (3 × 1 l.). The combined petroleum ether layers and the aqueous methanol layer were evaporated to give D (24 g) and E (25 g), respectively.

Fraction E was subjected to column chromatography (SilicAR, 940 g) and eluted with chloroform followed by chloroform containing increasing amounts of methanol. Elution with chloroform gave fraction F (3.8 g) and elution with 2% methanol in chloroform gave fraction G (3.5 g).

Glaucarubinone (4). Fraction G was further fractionated by column chromatography on SilicAR (175 g) with 2% ethanol in dichloromethane as eluent, to yield a residue which gave needles from acetone–hexane (**4**, 1.07 g, 0.05%). The material was identified by comparison of its melting point, $[\alpha]$D, uv, ir, and nmr spectra with those reported.[8]

2′-Acetylglaucarubinone (5). Fraction F was subjected to column chromatography on SilicAR (760 g). Elution with 1% isopropyl alcohol in methylene chloride gave a two-component mixture (H, 895 mg), while elution with 2% isopropyl alcohol in methylene chloride gave, after crystallization from acetone–hexane, **5** (0.20 g, 0.01%): mp 170–172°; $[\alpha]^{23}$D +77.0° (*c* 0.10, $CHCl_3$) (lit.[9] mp 173°, $[\alpha]$D +74°); uv max (EtOH) λ (ϵ) 240 (10,700) nm; ir (KBr) 2.82, 2.93, 3.05, 5.75, 5.98, 8.78 μ; nmr ($CDCl_3$) τ 9.03 (3 H, t, J = 7 Hz, CH_2CH_3), 8.87 (3 H, d, J = 6 Hz, 13-CH_3), 8.79 (3 H, s, 10-CH_3), 8.38 (3 H, s, 2′-CH_3), 7.99 (3 H, s, 4-CH_3), 7.93 (3 H, s, $C(=O)CH_3$), 6.41 (1 H, m, 12-H), 6.38, 6.12 (each 1 H, d, J = 8 Hz, CH_2O), 6.01 (1 H, s, 9-H), 5.32 (1 H, br s, 7-H), 4.90 (1 H, d, J = 12 Hz, 15-H), 3.95 (1 H, br s, 3-H); mass spectrum 536 (M^+), 518, 394, 247, 151, 43.

Anal. Calcd for $C_{27}H_{36}O_{11}$: C, 60.43; H, 6.76. Found: C, 60.30; H, 6.81.

Ailanthinone (6). Separation of the remaining two-component mixture (H) was effected with a SilicAR column (90 g), eluting with ether–benzene (1:1). The first material from the column was crystallized from acetone–hexane as needles (**6**, 0.008%, 0.17 g): mp 227–230°; $[\alpha]^{27}$D +90.0° (*c* 0.10, $CHCl_3$) (lit.[9] mp 231°, $[\alpha]$D 88°); uv max (EtOH) λ (ϵ) 239 (11,550) nm; ir (KBr) 2.82, 5.70, 5.74, 5.93, 8.18, 9.4 μ; nmr ($CDCl_3$) τ 9.03 (3 H, t, J = 7 Hz, CH_2CH_3), 8.88 (3 H, d, J = 6 Hz, 13-CH_3), 8.83 (3 H, d, J = 7 Hz, 2′-CH_3), 8.80 (3 H, s, 10-CH_3), 7.99 (3 H, s, 4-CH_3), 7.25 (1 H, s, 9-H), 6.45 (1 H, d, J = 3 Hz, 12-H), 6.34, 6.04 (each 1 H, d, J = 8 Hz, CH_2O), 5.91 (1 H, s, 1-H), 5.36 (1 H, br s, 7-H), 4.41 (1 H, d, J = 11 Hz, 15-H), 3.85 (1 H, br s, 3-H); mass spectrum *m/e* 478 (M^+), 394, 377, 247, 151, 85, 57.

Anal. Calcd for $C_{25}H_{34}O_9$: C, 62.75; H, 7.16. Found: C, 62.78; H, 7.27.

Dehydroailanthinone (1). Continued column chromatography of fraction H by elution with ether–benzene (1:1) gave, after further purification by ptlc (ChromAR, 2% isopropyl alcohol in dichloromethane), dehydroailanthinone (**1**, 270 mg, 0.015%) as a colorless glass: $[\alpha]^{23}$D +39.6° (*c* 0.24, $CHCl_3$); uv max (EtOH) λ (ϵ) 238 (10,900) nm; ir (KBr) 3.08, 5.71, 6.00, 8.45, 9.5 μ; nmr (pyridine-d_5) τ 9.05 (3 H, t, J = 7 Hz, CH_2CH_3), 8.85 (3 H, d, J = 7 Hz, 2′-CH_3), 8.50 (3 H, s, 10-CH_3), 8.26 (3 H, br s, 4-CH_3), 6.43 (1 H, s, 9-H), 6.32, 5.92 (each 1 H, d, J = 8 Hz, CH_2O), 5.79 (1 H, s, 1-H), 5.48 (1 H, s, 12-H), 5.25 (1 H, br s, 7-H), 4.84, 4.70 (each 1 H, d, J = 2 Hz, $=CH_2$), 3.95 (1 H, br s, 3-H), 3.67 (1 H, d, J = 12 Hz, 15-H); mass spectrum *m/e* 476 (M^+), 458, 447, 432, 392, 330, 247, 151, 85, 57.

Anal. Calcd for $C_{25}H_{32}O_9$: C, 63.01; H, 6.77. Found: C, 62.79; H, 6.83.

$\Delta^{13,18}$-Glaucarubolone (3) from Dehydroalilanthinone (1). A solution of dehydroailanthinone (**1**, 25 mg, 0.0525 mmol) in sodium hydroxide (1*N*, 0.4 ml) was allowed to stand at room temperature for 30 min. The mixture was neutralized (5% hydrochloric acid), evaporated to dryness, and extracted with methanol. The alcohol solubles were submitted to preparative tlc (ChromAR, 5% methanol–chloroform) and the major component was crystallized from methanol to give $\Delta^{13,18}$-glaucarubolone (**3**, 7.4 mg, 36%). Recrystallization from methanol gave an analytical sample: mp 224–226° (lit.[9] mp 230°); $[\alpha]^{24}$D +55.0° (*c* 0.20, MeOH); uv max (EtOH) λ (ϵ) 239 (8850) nm; ir (KBr) 2.80, 2.93, 5.79, 5.94, 8.27, 9.52 μ; nmr ($CDCl_3$) τ 8.43 (3 H, s, 10-CH_3), 8.22 (3 H, s, 4-CH_3), 6.39 (1 H, s, 9-H), 6.31 (1 H, s, 12-H), 6.23, 5.87 (each 1 H, d, J = 9 Hz, CH_2O), 5.53 (1 H, s, 1-H), 5.29 (1 H, br s, 7-H), 4.59 (1 H, d, J = 11 Hz, 15-H), 4.52, 4.48 (each 1 H, br s, $=CH_2$), 3.85 (1 H, br s, 3-H); mass spectrum *m/e* 392 (M^+), 374, 248, 151.

Anal. Calcd for $C_{20}H_{24}O_8 \cdot H_2O$: C, 58.53; H, 6.39. Found: C, 58.51; H, 6.61.

Dehydroailanthinone 1-Methyl Ether (2). A solution of dehydroailanthinone (**1**, 30 mg, 0.063 mmol) in methanol (5 ml) was treated with excess ethereal diazomethane for 5 hr. The solution was evaporated and submitted to preparative tlc on ChromAR with 3% methanol–chloroform as eluent, to give a slightly yellow crystalline product. Recrystallization from acetone–hexane gave dehydroailanthinone 1-methyl ether (**2**, 14 mg, 45%): mp 272–273.5°; $[\alpha]^{26}D$ −8° (*c* 0.11, $CHCl_3$); uv max (EtOH) λ (ε) 239 (11,650) nm; ir (KBr) 2.86, 3.08, 5.82, 5.95, 8.30, 13.3 μ; nmr ($CDCl_3$) τ 9.04 (3 H, t, *J* = 7 Hz, CH_2CH_3), 8.81 (3 H, d, *J* = 7 Hz, 2′-CH_3), 8.73 (3 H, s, 10-CH_3), 8.02 (3 H, s, 4-CH_3), 7.06 (1 H, s, 9-H), 6.45, 6.07 (each 1 H, d, *J* = 8 Hz, CH_2O), 6.27 (1 H, s, 1-H), 6.22 (3 H, s, OCH_3), 5.94 (1 H, s, 12-H), 5.40 (1 H, br s, 7-H), 4.80, 4.62 (each 1 H, s, =CH_2), 4.28 (1 H, d, *J* = 12 Hz, 15-H), 3.94 (1 H, br s, 3-H); mass spectrum *m/e* 490 (M^+), 390, 261, 229, 165, 135, 85, 51.

Anal. Calcd for $C_{26}H_{34}O_9 \cdot 0.5H_2O$: C, 62.50; H, 7.06. Found: C, 62.51; H, 7.02.

Registry No.—**1**, 53683-70-8; **2**, 53683-71-9; **3**, 53683-72-0; **4**, 1259-86-5; **5**, 33957-83-4; **6**, 53683-73-1.

References and Notes

(1) Tumor Inhibitors. 101. Part 100 is ref 2.

(2) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).

(3) This investigation was supported by grants from the National Cancer Institute (CA-11718) and American Cancer Society (CI-102J), and by a contract with the Division of Cancer Treatment, National Cancer Institute (NO1-CM-12099).

(4) S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **38**, 178 (1973).

(5) The stem bark was collected in Ghana in March 1973. The authors acknowledge with thanks receipt of dried plant material from Dr. R. E. Perdue, Jr., U.S. Department of Agriculture, in accordance with the program developed by the National Cancer Institute.

(6) Tumor-inhibitory activity and cytotoxicity were assayed under the auspices of the National Cancer Institute, by the procedures described by R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott [*Cancer Chemother. Rep., Part 3*, **3**, 1 (1972)].

(7) Dehydroailanthinone showed significant antileukemic activity against P-388 lymphocytic leukemia in the mouse over a 50- to 100-fold dosage range. 2′-Acetylglaucarubinone, glaucarubinone, and ailanthinone also showed significant inhibitory activity against P-388. All four compounds showed cytotoxicity (ED_{50}) against KB cell culture at 10^{-2}–10^{-3} μg/ml.

(8) A. Gaudemer and J. Polonsky, *Phytochemistry*, **4**, 149 (1965).

(9) J. Polonsky, *Fortschr. Chem. Org. Naturst.*, **30**, 101 (1973).

(10) Reported by J. Moron, J. Rondest, and J. Polonsky [*Experientia*, **22**, 511 (1966)] as compound F "which was probably 15-hydroxyailanthone" ($\Delta^{13,18}$-glaucarubolone). The compound has not been fully characterized, but its mp was reported in ref 9.

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The Structure of Abresoline

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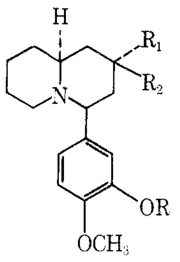
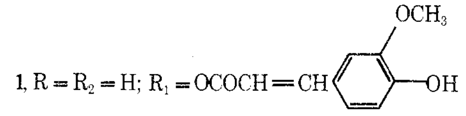
The alkaloids of *Heimia salicifolia* (Lythraceae) are a series of compounds which contain a quinolizidine nucleus substituted at positions 2 and 4 by a 12-membered lactone ring including a biphenyl grouping. The structures of the alkaloids are based upon chemical correlations[1–8] with lythrine (**2**) whose structure was assigned by X-ray crystallography.[9] Furthermore, there have been recent syntheses[10,11] of methyldecinine, the dihydromethyl ether of **2**, and of several lythraceous alkaloids containing biphenyl ether groupings[12–15] not found in the alkaloids of *Heimia*.

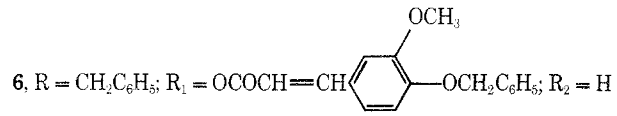
A minor alkaloid, $C_{26}H_{31}NO_6$, for which we propose the name abresoline (**1**), was isolated as a noncrystalline solid from *H. salicifolia* in very low yield. The uv spectrum, which is unlike those of the other *Heimia* alkaloids,[16] exhibits two maxima at 284 and 323 nm; the two transitions are readily correlated with those of *trans*-ferulic acid (**3**)[17] and the quinolizidol (**4**),[18] which are at 236 and 322 nm and 282 nm, respectively. Base hydrolysis of the alkaloid gave **3** and **4**, and the presence of these two units in the structure of **1** could also be inferred from the mass spectrum of the alkaloid, which contained strong transitions at *m/e* 276 and 177 corresponding to the fragment ions **4** and **3**. The ir spectrum of **1** has strong bands at 1706 and 2793 cm^{-1}, the first assigned to an ester carbonyl and the second to the Bohlman bands,[19] indicative of a *trans*-quinolizidine. The nmr spectrum of abresoline is also fully compatible with the assigned structure. Thus, typical upfield absorptions are seen for the quinolizidine ring protons; H-4 appears as a quartet (J_{aa} = 10 Hz, J_{ae} = 1 Hz) supporting the trans configuration of the ring and the axial orientation of the proton.[16,20] H-2 is seen at 5.18 ppm with a half-height width of 8 Hz indicating its equatorial orientation.[16,21,22] The olefinic protons at 6.36 (H-2‴) and 7.63 (H-1‴) form an AB quartet with *J* = 18 Hz; the trans geometry of the side chain is thus established.

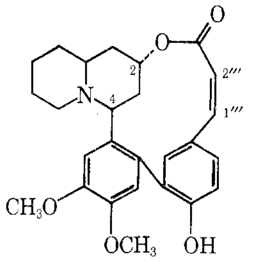



1, R = R_2 = H; R_1 = OCOCH=CH–C_6H_3(OCH₃)(OH)

4, R = R_2 = H; R_1 = OH

5, R_1R_2 = O; R = $CH_2C_6H_5$


6, R = $CH_2C_6H_5$; R_1 = OCOCH=CH–C_6H_3(OCH₃)–$OCH_2C_6H_5$; R_2 = H



2

Since the proposed formulation **1** represents a hitherto unknown structural variation in the series of *Heimia* alkaloids, its synthesis was undertaken. Pelletierine was condensed with 3-hydroxy-4-methoxybenzaldehyde to give the *trans*-quinolizidone, which was converted to its benzyl ether (**5**) and reduced with $NaBH_4$ to a mixture of epimeric alcohols. The alcohols could not readily be separated, and were therefore converted by transesterification with methyl benzyloxyferulate to a mixture of esters which was resolved by chromatography. The axial ester **6** was debenzylated with concomitant reduction to dihydroabresoline (**7**), which was chromatographically and spectroscopically identical with the reduced form of the natural alkaloid.

In 1966, it was proposed by Ferris and his coworkers[23] that the lythraceous alkaloids might be formed in nature by a series of steps very similar to those leading to synthetic abresoline. The biphenyl system would be derived from the *trans*-cinnamyl esters by isomerization and oxidative phenol coupling. The isolation of **1** certainly provides circumstantial evidence for the biosynthetic proposal, al-