The Isolation and Structural Elucidation of Bruceantin and Bruceantinol, New Potent Antileukemic Quassinoids from *Brucea antidysenterica*[1]

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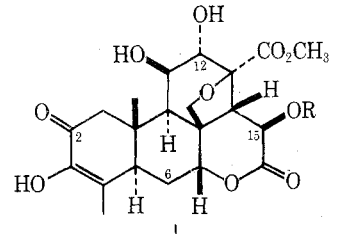
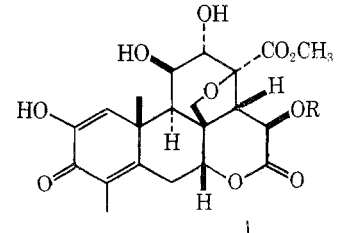
Received September 9, 1974

The isolation and structural elucidation of the new potent antileukemic principles, bruceantin (1) and bruceantinol (3), and the new companion quassinoids, bruceantarin (2), dehydrobruceantin (8), dehydrobruceantarin (9), dehydrobruceine B (10), dehydrobruceantol (11), and isobruceine B (12), are reported. Bruceantin (1), bruceantinol (3), and bruceantarin (2) were shown by hydrolysis to be *trans*-3,4-dimethyl-2-pentenoate, *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate, and benzoate esters, respectively, of bruceolide (5). The dehydro compounds were shown to have a 2-hydroxy-3-keto-4-methylcyclohexa-1,4-diene A ring, a feature new to the quassinoids. Isobruceine B (12) was shown to be an A-ring isomer of the known bruceine B (4).

Brucea antidysenterica Mill. is a Simaroubaceous tree which is used in Ethiopia in the treatment of cancer.[2] In the course of a continuing search for tumor inhibitors from plant sources, we found that an alcoholic extract of *Brucea antidysenterica*[3] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB), against Walker 256 intramuscular carcinosarcoma in the rat, and against P-388 lymphocytic leukemia in the mouse (PS). A preliminary communication[4] outlined the structural elucidation of the potent antileukemic (PS) principle, bruceantin (1), and the companion quassinoid, bruceantarin (2). Interest in the chemical and



1, R = CO-(C(CH3)=CHCH3)
2, R = COC6H5
3, R = CO-(C(CH3)(OH)-CH=CH2)
4, R = COCH3
5, R = H
6, R = COCH=C(CH3)C(OH)(CH3)2
7, R = COCH2CH(CH3)CH(CH3)2



8, R = CO-(C(CH3)=CHCH3)
9, R = COC6H5
10, R = COCH3
11, R = CO-(C(CH3)=CHCH3)
CH3—CHOH

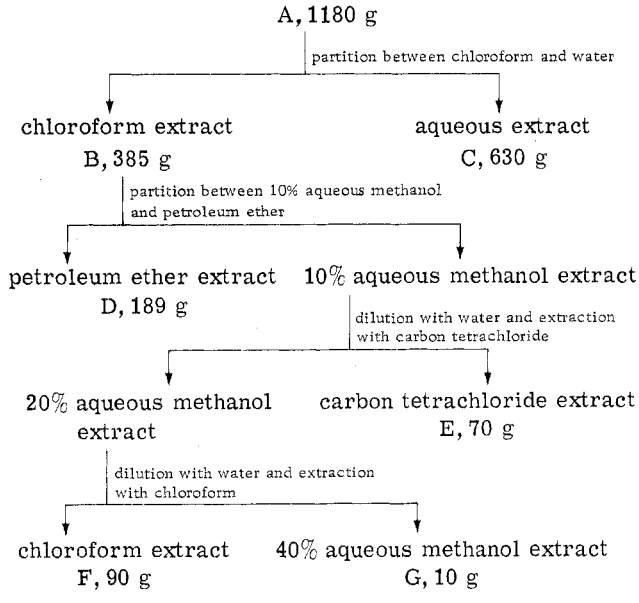
biological properties of bruceantin and related compounds has been heightened by recent findings. Thus, bruceantin also shows significant inhibitory activity against the L-1210 lymphoid leukemia, and against two solid murine tumor systems, the Lewis lung carcinoma and the B-16 melanocarcinoma.[5] Furthermore, bruceantin has been selected for toxicological investigation in preparation for clinical trials. It is the purpose of this paper to present in detail the isolation and structural elucidation of bruceantin (1), bruceantarin (2), the new potent antileukemic principle bruceantinol (3), and the companion quassinoids, dehydrobruceantin (8), dehydrobruceantarin (9), dehydrobruceine B (10), dehydrobruceantol (11), and isobruceine B (12).[6]

Table I
Activity of Fractions of *B. antidysenterica* against KB Tissue Culture

Fraction	ED_{50}, μg/ml	Fraction	ED_{50}, μg/ml
A	0.45	F	0.021
B	0.34	G	38.0
C	18.5	H	0.031
D	17.0	I	0.05
E	1.6	J	0.34

Fractionation (Chart I) of the alcohol extract, guided by assay (Table I) against KB tissue culture and PS leukemia

Chart I
Fractionation of the Active Extract from *Brucea antidysenterica*



in mice, revealed that the inhibitory activity was concentrated, successively, in the chloroform layer of a chloroform–water partition, the methanol layer of a 10% aqueous methanol–petroleum ether partition, the methanol layer of a 20% aqueous methanol–carbon tetrachloride partition and, finally, in the chloroform layer (F) of a chloroform–40% aqueous methanol partition. Column chromatography of fraction F on SilicAR yielded two KB and PS active fractions (H, I) upon elution with 1% methanol in chloroform. Continued elution with 2% methanol in chloroform gave a third KB-cytotoxic fraction (J).

Careful chromatography of fraction H on SilicAR with 20% ether in benzene as eluent gave bruceantin (1), as previously described.[4] Continued elution with 30% ether in benzene gave dehydrobruceantin (8) and isobruceine B (12). Column chromatography of fraction I on SilicAR, eluting with 30% ether in benzene, gave bruceantarin (2) and a fraction which on further separation by preparative tlc on ChromAR gave dehydrobruceantarin (9), bruceantinol (3) and dehydrobruceantol (11). Chromatography of fraction J in a similar manner gave the known bruceine B (4)[7] and dehydrobruceine B (10).

Bruceantin (1) and bruceantarin (2) displayed in their uv spectra the large bathochromic shift (from 280 to 330 nm) with alkali characteristic of diosphenols. The mass spectra of 1 and 2 displayed as primary fragmentation peaks corresponding to a loss of $C_7H_{11}O$ (*m/e* 438) and C_7H_5O (*m/e* 437), and base peaks corresponding to $C_7H_{11}O$ (*m/e* 111) and C_7H_5O (*m/e* 105), respectively. Except for the above-mentioned base peaks in the mass spectra of 1 and 2, peaks in the region from *m/e* 438 to 69 were almost identical with those present in the mass spectrum of bruceine B (4). Inspection of the nmr spectra of bruceantin (1), bruceantarin (2), and bruceine B (4) revealed that all three displayed peaks corresponding to an angular methyl group in the region τ 8.3–8.6, a vinyl methyl at τ 8.0–8.2, a methoxyl at τ 6.2–6.5, and a sharp one-proton doublet (J = 13 Hz) between τ 3.2 and 3.6 (assigned to H-15 in bruceine B (4)[7]). The major differences between the nmr spectra of bruceantin (1) and bruceine B (4) were the additional signals for 1 of a six-proton doublet (J = 6.5 Hz) at τ 8.88, a vinyl methyl signal at τ 7.82, and a vinyl proton singlet at τ 4.39. These data and the presence of the base peak at *m/e* 111 in the mass spectrum supported formulation of bruceantin as the 3,4-dimethyl-2-pentenoic acid ester (1) of bruceolide[7] (5).

Catalytic reduction of bruceantin (1) gave dihydrobruceantin (7), in which the double bond of the side-chain ester was reduced. That only the side-chain double bond was reduced was indicated by the uv spectrum, which still showed the diosphenol absorption and alkaline shift, and by the nmr spectrum, which showed no olefinic proton but a new three-proton doublet (J = 6.5 Hz) at τ 9.06. Mild alkaline hydrolysis of 7 gave bruceolide (5). In addition, alkaline hydrolysis of bruceantin (1) and esterification of the steam-distillable acid with diazoethane gave ethyl *trans*-3,4-dimethyl-2-pentenoate.[8] In the nmr spectrum of ethyl *cis*-3,4-dimethyl-2-pentenoate the vinyl methyl signal appears at τ 8.25, whereas the corresponding peak for the trans isomer occurs at τ 7.90. The peak attributed to the ester vinyl methyl in 1 appears at τ 7.82, indicative of trans stereochemistry in bruceantin (1).

The sharp one-proton doublet at τ 3.79 (J = 13 Hz) in the nmr spectrum of 1 indicated C-15 as the point of attachment of the ester side chain. The corresponding peak in the spectrum of dihydrobruceantin (7) appeared at τ 3.14 (J = 13 Hz) and in that of bruceine B (4) at τ 3.28 (J = 13 Hz).

In the nmr spectrum of bruceantarin (2), a complex A_2B_2X system centered at τ 2.3 was indicative of the presence of a benzoate group. In addition, the sharp one-proton doublet (J = 13 Hz) at τ 3.58 and the base peak at *m/e* 105 in the mass spectrum supported for bruceantarin the C-15 benzoate ester structure 2. The postulated structure was confirmed by mild alkaline hydrolysis of bruceantarin (2) to benzoic acid and bruceolide (5). In this way bruceantin (1) and bruceantarin (2) were shown to be esters (3,4-dimethyl-2-pentenoate and benzoate, respectively) of bruceolide (5). These two natural esters and the alcohol bruceolide gave a specific grey to black color when treated with ferric chloride on tlc. Bruceantinol (3) gave a very similar coloration with ferric chloride, while dehydrobruceantin (8), dehydrobruceantarin (9), dehydrobruceine B (10), and dehydrobruceantol (11) gave a distinctive brown color under the same conditions. Isobruceine B (12), however, did not react with ferric chloride.

The uv spectrum of bruceantinol (3) revealed the presence of an α,β-unsaturated ester in addition to a diosphenol; the latter was indicated by a bathochromic shift with alkali similar to bruceantin (1). The mass spectrum showed major ions at *m/e* 546, 438, 420, 297, 151, 127, and the base peak at 109, and the peaks 438–151 were almost identical with those of bruceantin (1). The presence of strong mass spectral ions at *m/e* 127 ($C_7H_{11}O_2$) and 109 (C_7H_9O), along with elemental analysis, supported the view that bruceantinol (3) is a $C_7H_{11}O_2$ ester of bruceolide (5). Treatment of bruceantinol (3) with hydrogen over a palladium catalyst resulted in reduction and hydrogenolysis, giving dihydrobruceantin (7), thus indicating that the bruceantinol side chain has the same carbon skeleton as that of bruceantin (1).

The nmr spectra of bruceantinol (3) and bruceantin (1) further confirmed their similarity. Both displayed resonances corresponding to the bruceolide (5) skeleton [*i.e.*, for the angular methyl, vinyl methyl, and methoxy methyl groups, and the H-15 proton (a one-proton doublet)], but, in addition, both showed signals for a vinyl proton and a vinyl methyl group assignable to the side chain. Instead of the isopropyl six-proton doublet of bruceantin (1), bruceantinol (3) displayed two methyl singlets at τ 8.60 and 7.98.

Based on these spectral data, the partial structure of bruceantinol could be written as in 6. This partial structure is identical with that reported for bruceine C.[7] The structural elucidation of the bruceine C side chain by Polonsky, *et al.*, involved ozonolysis of the side-chain double bond to give isopropyl methyl ketone. In this way, the center of geometric isomerism of the side-chain double bond was destroyed, and the exact structure of bruceine C (6) was not determined.

A comparison in our laboratory of bruceantinol (3) and a sample of bruceine C (6), kindly supplied by Dr. Polonsky, showed that the two compounds were different. Their nmr spectra, although very similar, differed in the peaks assigned to the terminal methyl groups of the side chain. The spectrum of bruceine C (6) displays a six-proton singlet at τ 8.59 for these two methyls, while that of bruceantinol (3) clearly shows them as two distinct three-proton singlets, when the spectra are taken in the same solvent at the same concentration. Furthermore, bruceine C (6) and bruceantinol (3) could be differentiated by mixture tlc in two different systems.

Alkaline hydrolysis of bruceantinol (3) at 0° followed by esterification with diazomethane gave the known bruceolide (5) and methyl *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate, identical with an authentic synthetic sample. The

synthetic ester was prepared from methyl *trans*-3-methyl-4-oxo-2-pentenoate by a Grignard reaction with methyl magnesium iodide.

The companion ferric chloride active compounds, dehydrobruceantin (**8**), dehydrobruceantarin (**9**), dehydrobruceine B (**10**), and dehydrobruceantol (**11**), displayed in their uv spectra a bathochromic shift with alkali, and gave an almost identical mass spectral fragmentation pattern from *m/e* 436 to 151. Inspection of the nmr spectra of the four compounds revealed that each displayed resonances corresponding to an angular methyl group (in the region τ 8.4–8.6), a vinyl methyl (at τ 8.0), a methoxy methyl (at τ 6.2–6.6), a one-proton doublet (J = 13 Hz, at τ 3.9–4.1), and a one-proton singlet (at τ 3.5). These spectral data supported the formulation that all four of these compounds are esters of the same alcohol.

The uv and nmr spectra are consistent with a diosphenol A ring as in **8**, where the downfield singlet can be assigned to the C-1 proton. A similar diosphenol system, but lacking a 4-methyl group, has been reported in a number of synthetic steroids,[9,10] which display the same uv maximum (254 nm). Dehydrobruceantin (**8**) forms a triacetate which neither reacts with ferric chloride nor gives a bathochromic shift with alkali in the uv spectrum, as expected for a blocked diosphenol.

In addition to the foregoing spectral data, dehydrobruceantin (**8**) displayed in the mass spectrum a parent ion at *m/e* 546 ($C_{28}H_{34}O_{11}$), a peak at 436, corresponding to the loss of $C_7H_{10}O$, and a base peak at 111 ($C_7H_{11}O$). Furthermore, the uv (225 nm) and the nmr (six-proton doublet, vinyl methyl and vinyl proton) spectra indicated that the side-chain ester of dehydrobruceantin (**8**) is identical with that of bruceantin (**1**), that is, a 3,4-dimethyl-2-pentenoate.

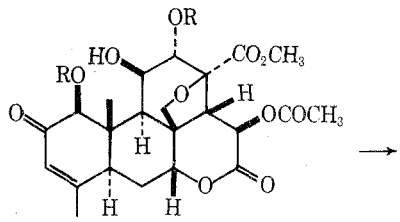
The relationship between bruceantin (**1**) and dehydrobruceantin (**8**) was confirmed through interconversion. Bruceantin (**1**) was oxidized with DDQ in benzene to give dehydrobruceantin (**8**), identical with the natural material.

In addition to the spectral data mentioned above for the dehydro alcohol, dehydrobruceantarin (**9**) and dehydrobruceine B (**10**) displayed in their mass spectra peaks corresponding to parent ions of $C_{28}H_{28}O_{11}$ (540) and $C_{23}H_{26}O_{11}$ (478) and base peaks of *m/e* 105 (C_7H_5O) and 43 (C_2H_3O), respectively. These data, together with the presence in the nmr of an A_2B_2X system of aromatic protons for dehydrobruceantarin and a three-proton acetate signal for dehydrobruceine B, confirmed their structures as **9** and **10**, respectively.

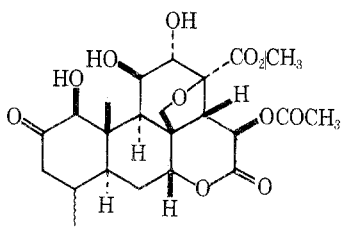
The molecular formula, $C_{28}H_{34}O_{12}$, was advanced for dehydrobruceantol (**11**) based on elemental and mass spectral analyses. The formula represents a $C_7H_{11}O_2$ ester of the dehydro skeleton alcohol. The ester, which was shown to be α,β-unsaturated by its uv spectrum, displayed nmr signals for vinyl methyls at τ 8.4 and 7.9, and for a methyl group (τ 8.62, doublet, J = 6 Hz) coupled to one proton (τ 4.64, quartet, J = 6 Hz), consistent with the presence of a methyl carbinol group. The geminal nature of the vinyl methyls in dehydrobruceantol (**11**) was proven by oxidation. Treatment of dehydrobruceantol (**11**) with excess ozone in aqueous dioxane gave acetone as the only volatile product. Structure **11** is consistent with all of the chemical and spectral properties of dehydrobruceantol.

Accompanying the ferric chloride active compounds was a KB-active and marginally PS-active crystalline material (**12**). The molecular formula $C_{23}H_{28}O_{11}$ was advanced on the basis of elemental and mass spectral analyses. That isobruceine B (**12**) was an A-ring isomer of bruceine B (**4**) was suggested by the similarity of mass spectral fragmentation pattern and nmr spectrum of **12** (which displayed signals for an angular methyl, a vinyl methyl, an acetate, and a carbomethoxy methyl, in addition to a downfield one-proton doublet) to those of **4**. The uv maximum (242 nm) was indicative of a β-disubstituted α,β-unsaturated ketone. In addition the upfield shift of the nmr signal for the vinyl methyl (from τ 8.1 in bruceine B (**4**) to 8.3 in isobruceine B (**12**)) and the appearance of two one-proton singlets at τ 5.93 and 4.08, assignable to the C-1 and C-3 protons, respectively, further supported the A-ring assignment as in **12**.

To prove the α-ketol nature of the A ring in isobruceine B (**12**), the compound was converted to a diosphenol. The double bond of **12** was catalytically reduced and the product, **14**, was subsequently oxidized with bismuth trioxide to the diosphenol, **15**. The product displayed a mass spectrum



12, R = H
13, R = $COCH_3$



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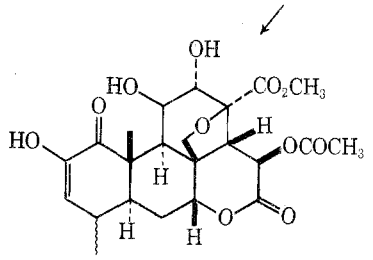

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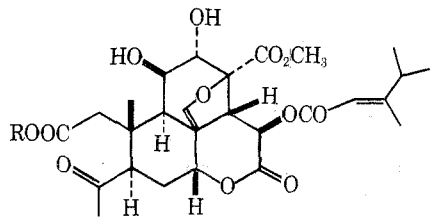
very similar to that of isobruceine B (**12**), and a uv spectrum and ferric chloride activity typical of diosphenols.

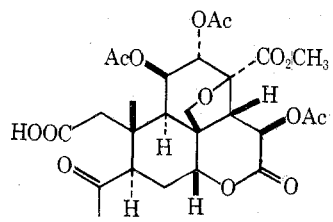
Acetylation of **12** with acetic anhydride–pyridine gave a 1,12-diacetate (**13**). This suggests that the C-1 alcohol is β in orientation and the resulting β-acetate causes sufficient steric hindrance to preclude acetylation at C-11. Moreover, all naturally occurring quassinoids with a C-1 alcohol have the 1-β configuration,[11] a fact which supports the presence of the 1-β-alcohol in **12**.

Column chromatography of fraction H, which had previously yielded bruceantin (**1**), gave a carboxylic acid (**16**). The same material was formed in varying, but small, amounts when pure bruceantin (**1**) was either aerated in a chloroform solution for 10 days, exposed to air and light on a tlc plate overnight, or even kept at low temperature and in the dark for a month or more. To further purify and characterize the acid (**16**), it was treated with diazomethane to give the methyl ether (**17**).

The molecular formula $C_{28}H_{38}O_{12}$ was advanced for **17**, based on elemental and mass spectral analyses. The presence of the bruceantin ester, *i.e.*, 3,4-dimethyl-2-pentenoate, in **16** and **17** was indicated by the uv maximum at



16, R = H
17, R = CH_3



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225 nm and by the presence of resonances in the nmr spectrum for a vinyl proton, a vinyl methyl, and an isopropyl group. The presence of other features in the nmr spectrum, such as signals corresponding to an angular methyl group, a carbomethoxy methyl group, and a C-15 proton, indicated that the B, C, and lactone rings were intact. The lack of ferric chloride activity and the absence of a conjugated ketone, indicated by the uv spectrum, suggested that the A ring of bruceantin (**1**) was changed significantly.

In the nmr spectrum, the signal for the C-4 methyl group (a vinyl methyl signal at τ 8.11 in bruceantin (**1**)) was shifted downfield to τ 7.77, consistent with the presence of a methyl ketone. The formation of a methyl ester, along with the spectral data mentioned above, is consistent with a cleaved A-ring acid as in **16**. The acid was evidently formed by oxidative cleavage of the 3,4 bond and loss of carbon-3. This compound is directly analogous to the product (**18**) of ozonolysis of bruceolide tetraacetate.[7]

The wood of stems and stem bark of *Brucea guineensis* G. Don, collected in Ghana in March, 1973, were extracted and fractionated by a procedure almost identical with that described for *B. antidysenterica*. Bruceantin (**1**), bruceantarin (**2**), bruceantinol (**3**), bruceine B (**4**), and dehydrobruceantin (**8**) were all isolated in yields comparable to those from *B. antidysenterica*.

The antileukemic activity of the bruceolide derivatives varies greatly with the nature of the ester substituent. Thus, bruceantin (**1**) and bruceantinol (**3**), which bear α,β-unsaturated esters, demonstrate potent antileukemic activity. Bruceantarin (**2**), which bears a benzoate ester, and dihydrobruceantin (**7**), which bears a saturated aliphatic ester moiety, both show moderate activity. Bruceine B (**4**), which bears the smaller acetate ester, and bruceolide (**5**), which bears no ester at all, show only marginal antileukemic activity. The limited results to data are consistent with the view that the ester moiety may serve as a carrier group involved in processes such as transport or complex formation. Investigations are in progress to determine the significance of the unsaturated ester, the diosphenol, and of other structural features in relation to the tumor inhibitory activity of bruceantin and bruceantinol.

Experimental Section

General Experimental. 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 JOEL 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 5% ferric chloride in 95% ethanol followed by vanillin (25% vanillin in 1:5 ethanol–concentrated sulfuric acid).

Brucea antidysenterica. **Extraction and Preliminary Fractionation.** Continuous extraction of 10 kg of *Brucea antidysenterica* dried ground stem bark was carried out at 72° with 95% ethanol in a Soxhlet extractor. The concentrated alcoholic extract (A, 1180 g) was partitioned between water (6 l.) and chloroform (6 l.). The water layer was washed with chloroform (6 l.) and the combined chloroform layers were evaporated to give a brown tar (B, 385 g). Evaporation of the water layer gave a brown tar (C, 630 g). Fraction B was partitioned between 10% aqueous methanol (6 l.) and petroleum ether (4 × 4 l.). Concentration of the petroleum ether layer gave a dark green tar (D, 189 g). The 10% aqueous methanol layer was diluted with water to 20% aqueous methanol and extracted with carbon tetrachloride (4 × 3.8 l.). The combined carbon tetrachloride layer was evaporated to give a green tar (E, 70 g). The 20% aqueous methanol layer was diluted with water to 40% aqueous methanol and extracted with chloroform (5 × 2.4 l.). The combined chloroform layer was evaporated to give a brown tar (F, 90 g) and the 40% aqueous methanol layer was evaporated to give a brown powder (G, 10 g). In this way all of the activity (KB and PS) was effectively concentrated in the final chloroform layer (fraction F). Fraction F was chromatographed on a column of SilicAR (5.4 kg) and eluted first with chloroform and then increasing amounts of methanol in chloroform. Fractions were combined on the basis of tlc similarity on ChromAR developed with 2:3 ether in benzene and visualized with ferric chloride and vanillin sprays. Elution with 1% methanol in chloroform gave a fraction (H, 8.1 g) which was active against PS and KB. Continued elution with 1% methanol in chloroform gave a PS and KB active fraction (I, 4.8 g) and elution with 2% methanol in chloroform gave a fraction (J, 3.6 g) active against KB.

Bruceantin (1). Careful column chromatography of fraction H on SilicAR (600 g) with benzene as eluent followed by benzene containing increasing amounts of ether gave, in the fractions eluted with 20% ether in benzene, bruceantin (**1**, 2.0 g, 0.02%): mp 225–226° (from ether[12]); $[\alpha]^{25}D$ −43° (*c* 0.31, pyridine); uv max (EtOH) λ (ϵ) 280 (8680), 221 (18,000) nm; uv max (EtOH + NaOH) λ (ϵ) 328 (7290), 221 (28,600) nm; ir (KBr) 2.90, 5.76, 6.05, 6.13, 8.70, 9.45 μ; nmr ($CDCl_3$) τ 8.88 (6 H, d, J = 6.5 Hz, $CH(CH_3)_2$), 8.56 (3 H, s, 10-CH_3), 8.11 (3 H, br s, 4-CH_3), 7.82 (3 H, s, $CH{=}C(CH_3)$), 7.29 (1 H, br m, OH), 6.47 (1 H, br s, OH), 6.24 (3 H, s, OCH_3), 4.29 (1 H, br s, $OCOCH{=}C$), 3.87 (1 H, br s, OH), 3.79 (1 H, d, J = 13 Hz, 15-H); mass spectrum *m/e* 548 (M^+), 438, 420, 402, 297, 151, 111.0819 (calcd for $C_7H_{11}O$, 111.0809).

Anal. Calcd for $C_{28}H_{36}O_{11}$: C, 61.30; H, 6.62. Found: C, 61.45; H, 6.65.

Dehydrobruceantin (8). Continued column chromatography of fraction H by elution with 30% ether in benzene gave dehydrobruceantin (**8**, 375 mg, 0.003%): $[\alpha]^{25}D$ +79.0° (*c* 0.62, pyridine); uv max (EtOH) λ (ϵ) 259 (8900), 225 (12,000) nm; uv max (EtOH + NaOH) λ (ϵ) 340 (1800), 263 (6900), 225 (15,000) nm; ir (KBr) 2.90, 5.78, 6.18, 8.07, 8.62, 9.45 μ; nmr ($CDCl_3$) τ 8.95 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 8.38 (3 H, s, 10-CH_3), 8.01 (3 H, s, 4-CH_3), 7.92 (3 H, s, $CH{=}C(CH_3)$), 6.32 (3 H, s, OCH_3), 4.46 (1 H, br s, $OCOCH{=}C$), 4.13 (1 H, d, J = 13 Hz, 15-H), 3.51 (1 H, s, 1-H); mass spectrum *m/e* 546 (M^+), 528.204 ($M^+ - H_2O$, calcd for $C_{28}H_{32}O_{10}$, 528.200), 436, 418, 400, 297, 151, 149, 111.079 (calcd for $C_7H_{10}O$, 111.081), 95.

Dehydrobruceantin was further characterized as its triacetate: mp 167–170° (crystallized from methylene chloride–ether); mass spectrum *m/e* 672 (M^+), 630, 472, 111, 43.

Anal. Calcd for $C_{34}H_{40}O_{14}$: C, 60.71; H, 5.99. Found: C, 60.49; H, 6.17.

Isobruceine B (12). Continued column chromatography of fraction H by elution with 30% ether in benzene gave a colorless glass (600 mg) which was crystallized from ether–methylene chloride to

afford needles (**12**, 360 mg, 0.004%): mp 243–246°; $[\alpha]^{25}D$ −36.2° (*c* 0.24, pyridine); uv max (EtOH) λ (ε) 242 (8850) nm; ir (KBr) 2.85, 5.75, 6.01, 6.08, 8.00, 8.20, 8.65, 9.42, 10.3 μ; nmr (pyridine-d_5) τ 8.74 (3 H, s, 10-CH_3), 8.30 (3 H, br s, 4-CH_3), 8.02 (3 H, s, $OCOCH_3$), 6.38 (3 H, s, OCH_3), 5.93 (1 H, s, 1-H), 4.08 (1 H, br s, 3-H), 3.52 (1 H, d, *J* = 13 Hz, 15-H); mass spectrum *m/e* 480 (M^+), 462, 438, 420, 402, 346, 314, 297, 151, 135, 95.

Anal. Calcd for $C_{23}H_{28}O_{11} \cdot H_2O$: C, 55.41; H, 6.06. Found: C, 54.96; H, 6.07.

Bruceantarin (2). Careful column chromatography of fraction I (4.8 g) on SilicAR (330 g) using benzene followed by benzene containing increasing amounts of ether gave, on elution with 30% ether in benzene, crystalline **2**. The crystalline fraction was treated with activated charcoal in chloroform and recrystallized from methylene chloride–benzene to give bruceantarin (**2**, 280 mg, 0.003%): mp 182–185°; $[\alpha]^{25}D$ −20.7° (*c* 0.60, pyridine); uv max (EtOH) λ (ε) 278 (7000), 231 (10,500) nm; uv max (EtOH + NaOH) λ (ε) 330 (4480), 230 (9030) nm; ir (KBr) 2.90, 5.78, 6.03, 6.08, 6.12, 7.88, 8.70, 9.00, 9.45, 13.8 μ; nmr ($CDCl_3$) τ 8.63 (3 H, s, 10-CH_3), 8.20 (3 H, br s, 4-CH_3), 6.56 (3 H, s, OCH_3), 3.58 (1 H, d, *J* = 13 Hz, 15-H), 2.60 (3 H, m, B_2X portion of A_2B_2X, *m*- + *p*-benzoate protons), 2.07 (2 H, d of d, *J* = 7.5, 1.5 Hz, A_2 of A_2B_2X, *o*-benzoate protons); mass spectrum *m/e* 542 (M^+), 437, 420, 402, 297, 151, 105, 77.

Anal. Calcd for $C_{28}H_{30}O_{11}$: C, 61.99; H, 5.57. Found: C, 62.06; H, 5.60.

Dehydrobruceantarin (9). Continued column chromatography of fraction I by elution with 30% ether in benzene gave a fraction rich in dehydrobruceantarin (100 mg). Preparative tlc on ChromAR developed with 2% isopropyl alcohol in methylene chloride gave dehydrobruceantarin (**9**, 40 mg, 0.0004%): $[\alpha]^{24}D$ +68.0° (*c* 0.15, pyridine); uv max (EtOH) λ (ε) 257 (8640), 231 (13,000) nm; uv max (EtOH + NaOH) λ (ε) 332 (2300), 265 (4500), 228 (17,850) nm; ir (KBr) 2.92, 5.77, 6.15, 7.93, 9.46 μ; nmr ($CDCl_3$) τ 8.37 (3 H, s, 10-CH_3), 7.99 (3 H, s, 4-CH_3), 6.58 (3 H, s, OCH_3), 3.85 (1 H, d, *J* = 13 Hz, 15-H), 3.48 (1 H, s, 1-H), 2.62 (3 H, m, B_2X of A_2B_2X, *m*- + *p*-benzoate protons), 2.08 (2 H, d of d, *J* = 7.5, 1.5 Hz, A_2 of A_2B_2X, *o*-benzoate protons); mass spectrum *m/e* 540 (M^+), 522.148 ($M^+ - H_2O$; calcd for $C_{28}H_{26}O_{10}$, 522.153), 418, 400, 151, 105.

Dehydrobruceantarin was further characterized as its triacetate: mp 181–184° (crystallized from benzene–ether); mass spectrum *m/e* 666 (M^+), 624, 372, 313, 105, 43.

Anal. Calcd for $C_{34}H_{34}O_{14} \cdot H_2O$: C, 59.64; H, 5.30. Found: C, 59.90; H, 5.00.

Bruceantinol (3). Continued column chromatography of fraction I by elution with 30% ether in benzene gave a fraction (490 mg) enriched in bruceantinol. Preparative tlc on ChromAR, with 2% isopropyl alcohol–methylene chloride as eluent, gave **3** (150 mg, 0.0015%): $[\alpha]^{24}D$ −14.5° (*c* 0.44, pyridine); uv max (EtOH) λ (ε) 278 (6650), 225 (14,100) nm; uv max (EtOH + NaOH) λ (ε) 328 (3230), 225 (10,000) nm; ir (KBr) 2.88, 5.79, 6.10, 6.95, 7.97, 9.46 μ; nmr ($CDCl_3$) 8.60, 7.98 (each 3 H, s, $C(OH)(CH_3)_2$), 8.43 (3 H, s, 10-CH_3), 8.15 (3 H, br s, 4-CH_3), 7.86 (3 H, s, $CH{=}C(CH_3)$), 6.18 (3 H, s, OCH_3), 4.23 (1 H, s, $OCOCH{=}C(CH_3)$), 3.74 (1 H, d, *J* = 13 Hz, 15-H); mass spectrum *m/e* 546.2106 ($M^+ - H_2O$; calcd for $C_{28}H_{34}O_{11}$, 546.2100), 438, 420, 402, 297, 151, 127.0765 (calcd for $C_7H_{11}O_2$, 127.0759), 109.

Anal. Calcd for $C_{28}H_{36}O_{12}$: C, 59.56; H, 6.43. Found: C, 59.50; H, 6.41.

Dehydrobruceantol (11). The preparative tlc which gave bruceantinol also gave dehydrobruceantol (**11**, 50 mg, 0.005%): $[\alpha]^{23}D$ +30.0° (*c* 0.11, $CHCl_3$); uv max (EtOH) λ (ε) 257 (8730), 219 (15,450) nm; uv max (EtOH + NaOH) λ (ε) 330 (1440), 262 (5900), 221 (22,000) nm; ir (KBr) 2.93, 5.73, 6.12, 8.00, 9.50 μ; nmr ($CDCl_3$) τ 8.62 (3 H, d, *J* = 6 Hz, $CH(OH)CH_3$), 8.50 (3 H, s, 10-CH_3), 8.27, 7.93 (each 3 H, s, $=C(CH_3)_2$), 8.01 (3 H, s, 4-CH_3), 6.16 (3 H, s, OCH_3), 4.64 (1 H, q, *J* = 6 Hz, $=CCH(OH)CH_3$), 4.14 (1 H, d, *J* = 13 Hz, 15-H), 3.49 (1 H, s, 1-H); mass spectrum *m/e* 544 ($M^+ - H_2O$), 526, 436, 418, 400, 151, 127, 109.

Anal. Calcd for $C_{28}H_{34}O_{12}$: C, 59.78; H, 6.09. Found: C, 59.65; H, 6.20.

Bruceine B (4). Careful chromatography of fraction J (3.6 g) on SilicAR (360 g) gave, on elution with 60% ether in benzene, a fraction rich in bruceine B. Further purification by preparative tlc (ChromAR, 1:1 ether–benzene) and crystallization from methylene chloride–ether gave needles (**4**, 83 mg, 0.0008%): mp 264–268°; mmp 262–264° [lit.[7] mp 262–266°; $[\alpha]D$ −77.2°]; $[\alpha]^{25}D$ −76.0° (*c* 1.01, pyridine); uv max (EtOH) λ (ε) 279 (8250) nm; uv max (EtOH + NaOH) λ (ε) 330 (7650)nm; ir (KBr) 2.90, 5.78, 6.04, 6.18, 7.90, 8.25, 9.45 μ; nmr (pyridine-d_5) τ 8.44 (3 H, s, 10-CH_3), 8.10 (3 H, br s, 4-CH_3), 7.96 (3 H, s, $OCOCH_3$), 6.30 (3 H, s, OCH_3), 3.26 (1 H, d, *J* = 13 Hz, 15-H); mass spectrum *m/e* 480 (M^+), 462, 438, 420, 402, 297, 151, 43.

Dehydrobruceine B (10). The mother liquors from the crystallization of bruceine B, which were subjected to ptlc (ChromAR, 1:1 ether–benzene), gave dehydrobruceine B (**10**, 8 mg, 0.00008%): $[\alpha]^{24}D$ +40.5° (*c* 0.20, chloroform); uv max (EtOH) λ (ε) 257 (8900) nm; uv max (EtOH + NaOH) λ (ε) 330 (3300), 264 (8170) nm; ir (KBr) 2.88, 5.75, 6.12, 7.28, 8.06, 9.51 μ; nmr ($CDCl_3$) τ 8.39 (3 H, s, 10-CH_3), 8.02 (3 H, s, $OCOCH_3$), 8.00 (3 H, s, 4-CH_3), 6.22 (3 H, s, OCH_3), 4.02 (1 H, d, *J* = 13 Hz, 15-H), 3.52 (1 H, s, 1-H); mass spectrum *m/e* 478 (M^+), 460, 436, 418, 201, 151, 43.

Anal. of high-resolution CIMS (Ar–H_2O). Calcd for $C_{26}H_{26}O_{11}$ + H: 479.160. Found: 479.154.

Keto Acid (16) and Methyl Ester (17). Column chromatography of fraction H, from which bruceantin had been isolated, was continued by elution with acetone. The fraction obtained was submitted to ptlc, with 4% isopropyl alcohol in methylene chloride as eluent, which gave **16** (36 mg): nmr ($CDCl_3$) τ 8.93 (6 H, d, *J* = 6 Hz, $CH(CH_3)_2$), 8.51 (3 H, s, 10-CH_3), 7.87 (3 H, s, $CH{=}C(CH_3)$), 7.75 (3 H, s, $COCH_3$), 6.26 (3 H, s, OCH_3), 4.35 (1 H, br s, $CH{=}C(CH_3)$), 3.71 (1 H, d, *J* = 13 Hz, 15-H); mass spectrum *m/e* 552 (M^+), 534, 442, 424, 111.

The acid **16** (10 mg) was methylated with ethereal diazomethane to give, after ptlc (ChromAR, 3% isopropyl alcohol in methylene chloride), **17** (7 mg): $[\alpha]^{23}D$ +44° (*c* 0.11, $CHCl_3$); uv max (EtOH) λ (ε) 220 (15,550) nm; ir (KBr) 2.73, 5.76, 6.09, 6.95, 8.25, 8.74, 13.3 μ; nmr ($CDCl_3$) τ 8.93 (6 H, d, *J* = 6 Hz, $=CH(CH_3)_2$), 8.74 (3 H, s, 10-CH_3), 7.85 (3 H, s, $CH{=}C(CH_3)$), 7.77 (3 H, s, $COCH_3$), 6.29, 6.25 (each 3 H, s, OCH_3), 4.36 (1 H, br s, $CH{=}C(CH_3)$), 3.87 (1 H, d, *J* = 12 Hz, 15-H); mass spectrum *m/e* 566 (M^+), 548, 535, 456, 111.

Anal. Calcd for $C_{28}H_{38}O_{12}$: C, 59.35; H, 6.76. Found: C, 59.38; H, 6.75.

Dihydrobruceantin (7). Bruceantin (**1**, 20 mg, 0.0365 mmol) was subjected to atmospheric pressure hydrogenation in absolute ethanol (5 ml) using 10% palladium on charcoal (20 mg) as catalyst. After 1 hr the catalyst was removed by filtration and the solvent was evaporated to afford a colorless glass (27 mg). Preparative tlc (ChromAR, 1:1 ether–benzene) and crystallization from ether afforded needles (**7**, 18.8 mg, 94%): mp 137–140°; $[\alpha]^{24}D$ −64.5° (*c* 2.90, pyridine); uv max (EtOH) λ (ε) 281 (10,300) nm; uv max (EtOH + NaOH) λ (ε) 332 (6450) nm; ir (KBr) 2.90, 5.77, 6.03, 6.13, 7.95, 8.70, 9.50 μ; nmr (pyridine-d_5) τ 9.23 (6 H, d, *J* = 7 Hz, $CH(CH_3)_2$), 9.06 (3 H, d, *J* = 6.5 Hz, $OCOCH_2CH(CH_3)$), 8.42 (3 H, s, 10-CH_3), 8.10 (3 H, br s, 4-CH_3), 6.22 (3 H, s, OCH_3), 3.14 (1 H, d, *J* = 13 Hz, 15-H); mass spectrum *m/e* 550 (M^+), 438, 420, 402, 392, 297, 151, 113.

Anal. Calcd for $C_{28}H_{38}O_{11}$: C, 61.08; H, 6.96. Found: C, 60.98; H, 6.94.

Bruceolide (5). A. From Dihydrobruceantin (7). To a cooled solution of 5 *N* sodium hydroxide (0.45 ml) and methanol (1.65 ml) was added dihydrobruceantin (**7**, 55 mg) and the reaction mixture was kept at −20° for 42 hr. The reaction mixture was neutralized with dilute hydrochloric acid and evaporated on the rotary evaporator. The residue was dissolved in chloroform and saturated sodium chloride solution and the aqueous layer was reextracted with chloroform. The combined chloroform layers were dried over magnesium sulfate and treated with excess ethereal diazomethane, and then evaporated to afford a colorless glass (22 mg). This material was applied to one ChromAR plate and developed with 5% isopropyl alcohol in methylene chloride to give, in the major band, 15.1 mg of a colorless foam, which was crystallized from ether–methylene chloride to afford bruceolide (**5**, 8.5 mg, 20%): mp 299–300°, mmp 299.5–300.5° (lit.[7] mp 300–302°); $[\alpha]^{25}D$ −92.5° (*c* 0.18, pyridine), (lit.[7] $[\alpha]D$ −95.4°); uv max (EtOH) λ (ε) 280 nm (8500); uv max (EtOH–NaOH) λ (ε) 330 nm (7750); ir (KBr) 2.85, 2.90, 5.78, 6.03, 6.13, 7.95, 8.23, 8.62, 9.35 μ; mass spectrum *m/e* 438 (M^+), 420, 402, 392, 297, 151, 91.

B. From Bruceine B (4). Bruceine B (**4**, 20 mg) was hydrolyzed and the product isolated as described above for dihydrobruceantin (**7**) to give crystalline bruceolide (**5**, 2.4 mg, 13%): mp 301–301.5°; identical by spectral comparisons with **5** described above.

C. From Bruceantarin (2). Bruceantarin (**2**, 37 mg) was hydrolyzed as described above for dihydrobruceantin (**7**) and after 42 hr was neutralized with dilute hydrochloric acid and evaporated to dryness. The residue was extracted with chloroform and the combined chloroform layer was dried over magnesium sulfate and evaporated to afford benzoic acid (5 mg, 60%): mp 122–123°; mmp

122–123°; identical by spectral comparisons with an authentic sample. A small amount of concentrated hydrochloric acid was added to the remaining aqueous layer and the aqueous layer was saturated with sodium chloride and then extracted with chloroform. The chloroform layer was dried over magnesium sulfate and treated with ethereal diazomethane to give, after evaporation of solvent, 4 mg of a colorless glass. This material was crystallized from ether–methylene chloride to give bruceolide (**5**, 2 mg, 7%): mp 298–300°; mmp 298–300°; identical by spectral comparisons with **5** described above.

Ethyl *trans*-3,4-Dimethyl-2-pentenoate. The ester was prepared essentially by the literature procedure using the Emmons reaction[8,13] and the mixture of cis:trans (10:90) isomers was separated by vapor phase chromatography on a 10% Carbowax 20M column (0.25 in. × 6 ft) at 95° to give pure ethyl *trans*-3,4-dimethyl-2-pentenoate: ir (film) 3.38, 5.83, 6.10, 8.13, 8.20, 8.55, 9.58 μ; mass spectrum *m/e* 156, 141, 113, 111, 95, 83, 67, 55, 41; nmr ($CDCl_3$) τ 8.97 (6 H, d, J = 7 Hz, $CH(CH_3)_2$), 8.76 (3 H, t, J = 7 Hz, OCH_2CH_3), 7.92 (3 H, br s, $CH{=}C(CH_3)$), 7.70 (1 H, septet, J = 7 Hz, $CH(CH_3)_2$), 5.95 (2 H, q, J = 7 Hz, OCH_2CH_3), 4.42 (1 H, br s, $OCOCH{=}CH(CH_3)$).

Ethyl *trans*-3,4-Dimethyl-2-pentenoate from Bruceantin (1). A solution of bruceantin (**1**, 140 mg) in a mixture of methanol (4.95 ml) and 5 *N* sodium hydroxide (1.35 ml) was stirred at room temperature overnight. The solvents were removed at aspirator pressure and the residue was acidified with dilute hydrochloric acid and then steam distilled. The steam distillate was saturated with sodium chloride and extracted with ether. The ether layer was dried over magnesium sulfate and treated with excess ethereal diazoethane. The solvent was removed at aspirator pressure and the residue (17 mg) was purified by preparative vapor phase chromatography (same conditions as above) to give ethyl *trans*-3,4-dimethyl-2-pentenoate (8 mg, 20%) identical with the synthetic sample.

Dihydrobruceantin (7) from Bruceantinol (3). Bruceantinol (**3**, 2 mg) was subjected to atmospheric pressure hydrogenation in absolute ethanol (2 ml) with 10% palladium on charcoal (4 mg) as catalyst. After 1 hr, the catalyst was removed by filtration, and the solvent was evaporated. The residue was submitted to ptlc (ChromAR, 3% isopropyl alcohol in methylene chloride) and gave dihydrobruceantin (**7**, 0.9 mg), identified by comparison of its ir, mass spectrum, tlc (5% isopropyl alcohol–methylene chloride on silica gel, and 1:1 ether–benzene on ChromAR) behavior, and high-pressure liquid chromatography (Corasil II, 1.5% methanol–methylene chloride) retention time with authentic **7** obtained from bruceantin.

Methyl *trans*-4-Hydroxy-3,4-dimethyl-2-pentenoate. 3-Methyl-4-oxo-2-pentenoic acid (Aldrich) was esterified with methanol containing 3% HCl. The resulting mixture of cis:trans (1:3) isomers was separated by vapor phase chromatography on a 10% Carbowax 20M column (0.25 in. × 6 ft) at 180° to give pure methyl *trans*-3-methyl-4-oxo-2-pentenoate: uv max (EtOH) λ (ϵ) 232 (12,500) nm; ir (KBr) 5.78, 5.90, 6.08, 6.97, 7.32, 9.59 μ; nmr ($CDCl_3$) τ 7.84 (3 H, d, J = 2 Hz, $C{=}C(CH_3)$), 7.67 (3 H, s, $C(=O)CH_3$), 6.28 (3 H, s, OCH_3), 3.53 (1 H, q, J = 2 Hz, $C{=}CH$); mass spectrum *m/e* 142 (M^+), 127, 111, 110, 99, 85, 67, 59, 43.

To methyl *trans*-3-methyl-4-oxo-2-pentenoate (71 mg, 0.5 mmol) in ether (5 ml) at 0° under nitrogen was added dropwise with stirring methyl magnesium iodide (177 μl, 2.82 *M* in hexane, 0.5 mmol, Alfa Inorganics). The mixture was maintained at 0° for 30 min, then allowed to warm to room temperature. Hydrochloric acid (5%, 5 ml) was added and the ether layer was separated. The aqueous layer was extracted twice with ether; the combined ether extracts were dried ($MgSO_4$) and evaporated to give a yellow oil. Purification by vpc, as above, gave methyl *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate, as a colorless liquid (50 mg, 63%): uv max (EtOH) λ (ϵ) 216 (14,000) nm; ir (KBr) 2.87, 5.81, 6.08, 6.97, 8.47, 9.65 μ; nmr ($CDCl_3$) τ 8.64 (6 H, s, $C(OH)(CH_3)_2$), 7.86 (3 H, d, J = 2 Hz, $C{=}C(CH_3)$), 7.82 (1 H, s, OH), 6.38 (3 H, s, OCH_3), 3.98 (1 H, q, J = 2 Hz, $C{=}CH$); mass spectrum *m/e* 158 (M^+), 143, 140, 115, 111, 83, 59, 43; vpc retention times: (a) 10.0 min (0.25 in. × 6 ft Carbowax on Chromosorb W, 180°, 60 ml/min gas flow); (b) 2.20 min (⅛ in. × 6 ft 3% SE-30 on 100–120 mesh Porapac 30, 100°, 40 ml/min gas flow).

Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.53; H, 8.83.

Methyl *trans*-4-Hydroxy-3,4-dimethyl-2-pentenoate and Bruceolide (5) from Bruceantinol (3). A solution of bruceantinol (**3**, 70 mg) in a mixture of methanol (2.4 ml) and sodium hydroxide (5*N*, 0.6 ml) was allowed to stand at 0° for 48 hr. After acidification (HCl), the aqueous layer was extracted with ether, salted (NaCl), and reextracted with ether. The combined ether extracts were dried ($MgSO_4$) and treated with ethereal diazomethane. Preparative tlc of the reaction mixture on ChromAR with 4% isopropyl alcohol in methylene chloride as eluent gave methyl *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate (10.7 mg, 55%), identical by ir, nmr, mass spectrum, and vpc retention times to the synthetic material. The ferric chloride active band from the ptlc gave crystalline bruceolide (**5**, 2.2 mg, 2%), identical in all respects with authentic bruceolide obtained from the hydrolysis of bruceantin.

Dehydrobruceantin (8) from Bruceantin (1). To bruceantin (**1**, 20 mg, 0.037 mmol) in dry benzene (2 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (10 mg, 0.044 mmol) and the mixture was heated under reflux for 6 hr. Evaporation of the solvent and ptlc of the residue (ChromAR, 2% isopropyl alcohol in methylene chloride) gave dehydrobruceantin (**8**, 8.8 mg, 44%), identical with natural dehydrobruceantin in nmr, ir, uv, $[\alpha]D$, and mass spectrum.

Ozonolysis of Dehydrobruceantol (11). Dehydrobruceantol (**11**) was ozonized and the volatile product identified by the method of Moore and Brown.[14] Thus, a solution of dehydrobruceantol (**11**, 0.6 mg) in methylene chloride (1.0 ml) was treated with excess ozone, and after addition of excess triphenylphosphine, the solution was submitted to vpc analysis. Acetone was identified as the only volatile product by comparison of vpc retention times (⅛ in. × 10 ft, 10% β,β'-oxidipropionitrile on Chromosorb B).

Isobruceine B Diacetate (13). To isobruceine B (**12**, 15 mg, 0.031 mmol) in pyridine (1 ml) was added acetic anhydride (1 ml), and the mixture was kept at room temperature for 3 days. The mixture was evaporated to dryness and the residue was submitted to ptlc (ChromAR, 2% isopropyl alcohol in methylene chloride). Crystallization of the major component from ether–methylene chloride gave needles (**13**, 11 mg, 63%): mp 264–267°; uv max (EtOH) λ (ϵ) 238 (12,200) nm; ir (KBr) 2.83, 5.73, 5.95, 7.30, 8.13, 9.65 μ; nmr ($CDCl_3$) τ 8.86 (3 H, s, 10-CH_3), 8.12 (3 H, s, 4-CH_3), 8.05, 7.97, 7.84 (each 3 H, s, $OCOCH_3$), 6.31 (3 H, s, OCH_3), 4.85 (1 H, d, J = 15 Hz, 15-H), 4.05 (1 H, br s, 3-H); mass spectrum *m/e* 564 (M^+), 522, 504, 489, 135, 95, 91, 60, 43.

Anal. Calcd for $C_{27}H_{32}O_{13}$: C, 57.44; H, 5.71. Found: C, 56.97; H, 5.96.

Diosphenol (15) from Isobruceine B (12). A solution of isobruceine B (**12**, 20 mg) in ethanol (20 ml) was subjected to atmospheric pressure hydrogenation for 15 hr using 5% palladium on charcoal (20 mg) as catalyst. The catalyst was removed by filtration and the solvent was evaporated *in vacuo*. The residue, after ptlc (ChromAR, 3% isopropyl alcohol in methylene chloride) and crystallization from methylene chloride–ether, gave dihydroisobruceine B (**14**, 15 mg, 74%): mp 294–296°; mass spectrum *m/e* 482 (M^+). Dihydroisobruceine B (**14**) was then oxidized by the method of Kupchan, *et al.*[15] To dihydroisobruceine B (**14**, 15 mg) in acetic acid (2 ml) was added bismuth(III) oxide, freshly prepared from bismuth subcarbonate (19.5 mg), and this mixture was heated at reflux for 30 min. The reaction mixture was cooled, diluted with water, and extracted three times with chloroform. The combined chloroform layers were dried ($MgSO_4$) and evaporated. Ptlc and crystallization of the major fraction gave the diosphenol **15** (0.9 mg): mp 184–187°; uv max (EtOH) λ (ϵ) 269 (7600) nm; uv max (EtOH + NaOH) λ (ϵ) 314 (4800) nm; mass spectrum *m/e* 480 (M^+), 462, 400, 325, 151, 43.

Registry No.—**1**, 41451-75-6; **2**, 41451-76-7; **3**, 53729-52-5; **4**, 25514-29-8; **5**, 25514-28-7; **7**, 41328-90-9; **8**, 53662-98-9; **8** triacetate, 53662-99-0; **9**, 53663-00-6; **9** triacetate, 53663-01-7; **10**, 53730-90-8; **11**, 53663-02-8; **12**, 53663-03-9; **13**, 53663-05-1; **14**, 53663-04-0; **15**, 53663-06-2; **16**, 53663-07-3; **17**, 53663-08-4; ethyl *trans*-3,4-dimethyl-2-pentenoate, 21016-44-4; ethyl *cis*-3,4-dimethyl-2-pentenoate, 21016-45-5; methyl *trans*-4-hydroxy-3,4-dimethyl-2-pentenoate, 53663-09-5; 3-methyl-4-oxo-2-pentenoic acid, 53663-10-8; methyl *trans*-3-methyl-4-oxo-2-pentenoate, 53663-11-9; methyl *cis*-3-methyl-4-oxo-2-pentenoate, 53663-12-0.

References and Notes

(1) (a) Tumor Inhibitors. 100. Part 99: S. M. Kupchan, R. L. Baxter, M. F. Ziegler, P. M. Smith, and R. F. Bryan, submitted for publication. (b) 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 National Cancer Institute (N01-CM-12099).
(2) J. L. Hartwell, *Lloydia*, **34**, 221 (1971).
(3) Stem bark was collected in Ethiopia in June, 1971. Leaves and the

wood of stems from Ethiopia also yielded active extracts. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.
(4) S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **33**, 178 (1973).
(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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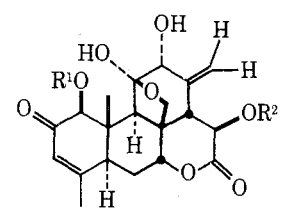
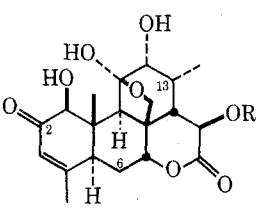
Received September 9, 1974

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, $R^1 = H$; $R^2 = COCH(CH_3)C_2H_5$
2, $R^1 = CH_3$; $R^2 = COCH(CH_3)C_2H_5$
3, $R^1 = R^2 = H$



4, $R = COC(OH)(CH_3)C_2H_5$
5, $R = COC(OAc)(CH_3)C_2H_5$
6, $R = COCH(CH_3)C_2H_5$
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