Structural Requirements for Biological Activity among Antileukemic Glaucarubolone Ester Quassinoids[1]

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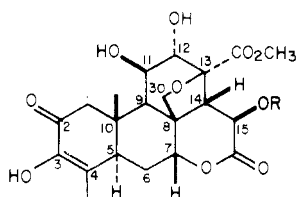
A C-15 ester substituent is required for significant antileukemic activity among the glaucarubolone ester quassinoids, and variations in the ester group are not accompanied by particularly marked changes in antileukemic activity. Unsaturation at the 3,4 position is advantageous for optimal activity, and hydrogenation of this double bond results in marked diminution in both cytotoxicity toward KB cells in tissue culture and inhibitory activity against the P-388 lymphocytic leukemia in mice.

Earlier work in this laboratory on quassinoids from the genus *Brucea* in the plant family Simaroubaceae led to the isolation of bruceantin (**1**)[2] and bruceantinol (**2**),[3] which showed potent antileukemic activity against the P-388 lymphocytic leukemia in the mouse (PS). Bruceantin (**1**) and bruceantinol (**2**) were shown to be α,β-unsaturated esters (i.e., 3,4-dimethyl-2-pentenoate and 4-hydroxy-3,4-dimethyl-2-pentenoate, respectively) of bruceolide (**5**).



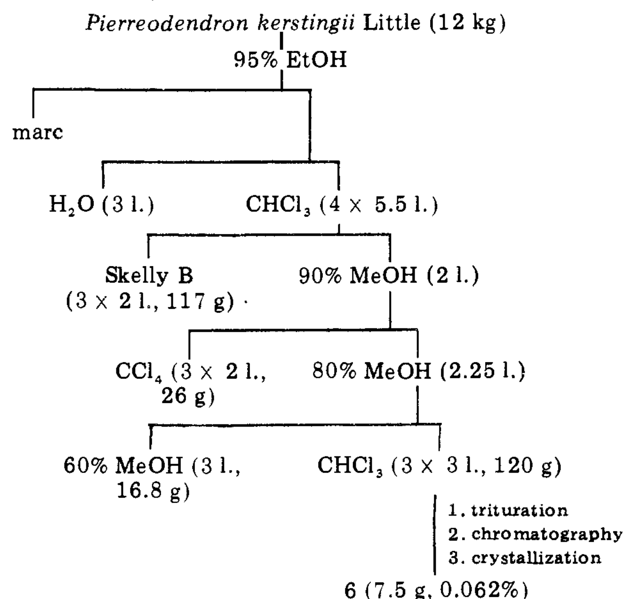
1, R = (E)COCH=C(CH$_3$)CH(CH$_3$)$_2$
2, R = (E)COCH=C(CH$_3$)C(OH)(CH$_3$)$_2$
3, R = COC$_6$H$_5$
4, R = COCH$_3$
5, R = H

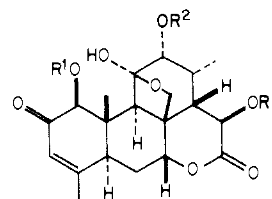
The companion benzoate ester of bruceolide, bruceantarin (**3**), showed moderate activity against the P-388 leukemia; the acetate ester, bruceine B (**4**), showed only marginal activity against this tumor system, and the parent quassinoid, bruceolide (**5**), was inactive. The most potent of the naturally occurring bruceolide esters were thus compounds having unsaturated ester side chains. Moreover, reduction of the unsaturated ester in bruceantin gave dihydrobruceantin, a derivative with diminished antileukemic activity.

The interesting structure–activity correlations among the bruceolide esters led us to investigate in more detail a second series of quassinoids. Glaucarubinone (**6**),[4] a saturated ester of glaucarubolone (**12**),[4] showed moderate activity against the P-388 system. Glaucarubinone was isolated from *Pierreodendron kerstingii* (Simaroubaceae) as shown in Scheme I, and its side chain and A ring were modified chemically to study the role which each plays in the antileukemic activity of the glaucarubolone esters.

To determine whether unsaturation in the ester moiety might enhance activity, both an acyclic α,β-unsaturated ester and a benzoate ester were prepared. The α,β-unsaturated ester (**10**) of glaucarubolone was synthesized by a sequence involving dehydration of the glaucarubinone side chain. Initial attempts at dehydration of glaucarubinone triacetate (**14**)[4] gave a mixture of many products. When glaucarubinone 1,12-diacetate (**9**), prepared by partial hydrolysis of **14**, was treated with thionyl chloride in pyridine and then with potassium hydroxide in aqueous alcohol, the desired glaucarubolone 15-tiglate (**10**) was obtained. Elemental analysis and mass spectrometry confirmed the formula $C_{25}H_{32}O_9$ for **10**. The details of the spectrum and the presence of mass spectral fragment ions at *m/e* 247 and 151, which are common ions in the mass spectra of glaucarubolone and related compounds,[5] confirmed that the glaucarubolone skeleton was intact. The presence of a tiglate ester was shown by the loss in the mass spectrum of 100 amu [HOOCC(CH$_3$)=CHCH$_3$] from the parent ion and by the appearance of peaks at *m/e* 83 [O≡CC(CH$_3$)=CHCH$_3$]$\cdot^+$ and 55 (CH$_3$C=CHCH$_3$)$\cdot^+$. Furthermore, the presence in the NMR spectrum of resonances for two vinyl methyl groups (at τ 8.18 and 8.13) and a vinyl proton (at τ 3.04) supported the characterization of the ester as a tiglate. The resonance of the vinyl proton of the isomeric angeloyl residue occurs at a very different chemical shift (τ 3.91).[6]

Scheme I. Isolation of Glaucarubinone (6) from *P. kerstingii*





6, R^1 = R^2 = H; R^3 = COC(OH)(CH$_3$)CH$_2$CH$_3$
7, R^1 = R^2 = H; R^3 = COCH(CH$_3$)CH$_2$CH$_3$
8, R^1 = R^2 = H; R^3 = COC(OCOCH$_3$)(CH$_3$)CH$_2$CH$_3$
9, R^1 = R^2 = COCH$_3$; R^3 = COC(OH)(CH$_3$)CH$_2$CH$_3$
10, R^1 = R^2 = H; R^3 = (E)COC(CH$_3$)=CHCH$_3$
11, R^1 = R^2 = H; R^3 = COC$_6$H$_5$
12, R^1 = R^2 = R^3 = H

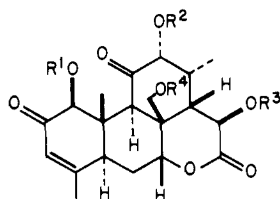
Glaucarubolone 15-benzoate (**11**) was prepared by a sequence involving specific hydrolysis of the tribenzoate (**13**). Treatment of glaucarubolone (**12**) with excess benzoyl chloride in pyridine gave glaucarubolone tribenzoate (**13**), as indicated by mass spectrometry and elemental analysis. That the alcohol at C-12 was not esterified was indicated

Table I. Antileukemic Activity of the Quassinoid Derivatives toward the P-388 System[9]

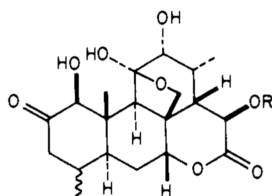
Compd	Dose, mg/kg	Survival	Wt diff	T/C, %	Compd	Dose, mg/kg	Survival	Wt diff	T/C, %
3	1.0	1/6	-4.2		11	8.0	6/6	-0.2	137
	0.50	5/6	-3.7	161		4.0	6/6	-0.5	151
	0.25	5/6	-0.6	130		2.0	6/6	-1.2	161
	0.12	6/6	-1.1	130		1.0	5/6	-0.3	134
	0.06	6/6	-2.0	129		0.50	6/6	-1.4	139
	0.03	6/6	0.0	86		0.25	6/6	-1.1	131
						0.12	6/6	0.0	122
7	8.0	4/6	-1.7	95	12	32.0	4/6	-3.3	53
	4.0	5/6	-1.3	127		16.0	6/6	-1.8	117
	2.0	6/6	-0.8	148		8.0	6/6	-1.9	108
	1.0	6/6	-1.7	138		4.0	6/6	-1.1	117
	0.50	6/6	-0.6	148		2.0	6/6	-0.5	124
	0.25	6/6	-0.6	138		1.0	6/6	-1.7	86
8	5.0	3/4	-1.6	123	15[a]	32.0	6/6	-1.6	139
	2.5	3/4	-1.0	142		16.0	6/6	-0.8	119
	1.2	4/4	-1.4	157		8.0	6/6	-1.2	117
	0.6	3/4	-0.9	142		4.0	6/6	-0.6	112
	0.3	4/4	-1.5	119		2.0	6/6	-0.1	121
						1.0	6/6	-0.4	119
10	8.0	6/6	-1.1	100	16[a]	32.0	6/6	-1.0	129
	4.0	6/6	-1.2	110		16.0	6/6	-1.3	153
	2.0	6/6	-1.8	147		8.0	6/6	-1.2	122
	1.0	5/6	-0.6	147		4.0	6/6	-1.0	127
	0.50	6/6	-0.5	125		2.0	6/6	-0.6	127
	0.25	6/6	-0.5	114		1.0	6/6	-0.9	122

[a] Tested as an isomer mixture.

by the chemical shift of the C-12 proton in the NMR spectrum and by the downfield chemical shifts of the C-1, C-15, and C-30 protons. Hydrolysis of **13** with triethylamine in aqueous methanol for 16 h gave a clean conversion to glaucarubolone 15-benzoate (**11**). The chemical shift of the C-15 proton in the NMR spectrum confirmed the position of the benzoate, and the elemental analysis and mass spectrum supported the structure. The marked enhancement in the rate of solvolysis of the C-1 ester in **13** is noteworthy. It is conceivable that this solvolysis may be facilitated by intramolecular hydrogen bonding of the ester to the β-hydroxyl group of a hemiketal formed by attack of methanol on the C-11 ketone.[7,8] Steric hindrance by the α-oriented C-12 acetate in **14** to the approach of methanol to C-11 may interfere with analogous hemiketal formation.



13, $R^1 = R^3 = R^4 = COC_6H_5$; $R^2 = H$
14, $R^1 = R^2 = R^4 = COCH_3$; $R^3 = COC(OH)(CH_3)CH_2CH_3$



15, $R = COC_6H_5$
16, $R = COC(OH)(CH_3)CH_2CH_3$

Reduction of glaucarubolone 15-benzoate (**11**) using palladium on charcoal as catalyst gave a crystalline product which was characterized as dihydroglaucarubolone 15-benzoate (**15**) on the basis of elemental analysis and spectral data. Although chromatography in four other systems suggested that the product was homogeneous, chromatography in ethyl acetate–cyclohexane (1:1) showed the product to be a two-component mixture. The NMR spectrum revealed that the methyl resonance of the lower R_f component was not resolved in $CDCl_3$; addition of C_6D_6 to the $CDCl_3$ solution transformed the unresolved methyl resonance into a methyl singlet and two methyl doublets. The NMR spectrum of the higher R_f material (the major component) showed a methyl singlet and two methyl doublets in $CDCl_3$, and elemental analysis of this component corresponded to dihydroglaucarubolone 15-benzoate (**15**). The two products are produced in ca. 7:3 ratio of higher R_f to lower R_f material as judged by NMR spectroscopy. Similarly, reduction of glaucarubinone (**6**) gave a crystalline product which was characterized as dihydroglaucarubinone (**16**) by elemental analysis and spectral data and which was shown by TLC to be a two-component mixture. Again, the NMR spectra of the resulting products showed differences in the methyl region which were consistent with stereoisomer formation via attack on both the α and the β faces of ring A during reduction.

Table II. Cytotoxicity of the Quassinoid Derivatives toward KB Cells[9]

Compd	ED_{50}, $\mu g/ml$	Compd	ED_{50}, $\mu g/ml$
6	0.08	11	0.90
7	0.12	12	0.37
8	0.012	15[a]	9.0
10	0.26	16[a]	44.0

[a] Tested as an isomer mixture.

The requirement of an ester side chain for significant in vivo activity (Table I) is demonstrated by the testing results[9] for glaucarubinone (**6**) and glaucarubolone (**12**). Both are equally active in the inhibition of growth of KB cells (Table II). However, glaucarubolone is inactive toward the P-388 leukemia in mice. Variations of the ester side chain, as in **7**, **8**, **10**, and **11**, had little effect upon in vivo activity, suggesting that the ester may be involved primarily in transport. A marked decrease in antileukemic activity results from reduction of the A ring, indicating that

this portion of the molecule plays a more fundamental role in the antileukemic activity. Dihydro derivatives 15 and 16 were inactive toward KB and showed antileukemic activity approaching that of precursors 11 and 6 only at doses 30-fold greater. The results to date indicate that a C-15 ester is required for antileukemic activity among the glaucarubolone ester quassinoids and that variations in the ester group are not accompanied by particularly marked changes in antileukemic activity. Unsaturation at the 3,4 position is advantageous for optimal activity, and hydrogenation of this double bond results in marked diminution in both cytotoxicity toward cells in tissue culture and in vivo antileukemic activity.

Earlier studies have demonstrated the importance of electrophilic functions for the biological activity of other tumor-inhibitory natural products.[10] Cyclohexenone has been shown to react readily with nucleophiles.[11] The observed requirement of the intact ring A cyclohexenone moiety for optimal biological activity among the glaucarubolone ester quassinoids suggests that these compounds may also act by selective alkylation of growth-regulatory biological macromolecules.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Ultraviolet absorption spectra were determined on Beckmann Model DK-2A and Coleman Hitachi Model EPS-3T recording spectrophotometers. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. NMR 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., and satisfactory results for C and H were obtained. All thin-layer chromatography was carried out on commercially prepared plates (0.25 mm × 20 × 20 cm) and visualization was effected with short-wavelength uv, with iodine spray, or with ceric ammonium sulfate spray and heat. "ChromAR" refers to ChromAR 7GF plates by Mallinckrodt, and "silica gel" refers to silica gel F-254 plates by E.M. Reagents. Solvent removal, referred to as "concentration", was effected at aspirator pressure.

Isolation of Glaucarubinone (6), Ailanthinone (7), and 2'-Acetylglaucarubinone (8). Initial isolation of 6–8 was effected by the reported procedure.[12] However, an improved procedure (Scheme I) was developed to obtain the large amounts of glaucarubinone required for synthetic modification. The dried stem bark of *P. kerstingii* (12 kg) was extracted successively (Soxhlet, 6 and 15 h) with two portions of 95% ethanol (total, 64 l.). After concentration, the extract (1.24 kg) was partitioned between water (3 l.) and chloroform (4 × 5.5 l.). The concentrated chloroform layer was successively partitioned between 1:9 water–methanol (2 l.) and petroleum ether (bp 60–68°, 3 × 2 l.), 1:4 water–methanol (2.25 l.) and carbon tetrachloride (3 × 2 l.), and then 2:3 water–methanol (3 l.) and chloroform (3 × 3 l.). The final chloroform layer was concentrated (120 g) and triturated three times with ethyl acetate (500, 200, and then 100 ml). Concentration of the combined ethyl acetate extracts gave 60 g of tractable residue which was adsorbed on silica gel 60 (E.M. Reagents, 500 g) and eluted with ethyl acetate. The three 1-l. fractions immediately following a 350-ml forerun were combined, concentrated (45 g), and chromatographed on SilicAR CC-7 (Mallinckrodt, 1 kg) packed in chloroform. Fractions (2.2 l. each) 1–3 (chloroform), 4–5 (1% methanol–chloroform), and 6–13 (2% methanol–chloroform) were collected. Crystallization from acetone–hexane of the residue from fractions 7–9 gave 6 (6.5 g, 0.054%). Rechromatography (SilicAR CC-7, 1 kg) of the mother liquors from 6, followed by crystallization of the appropriate fractions, gave additional glaucarubinone (1.01 g, 0.0084%).

Glaucarubinone 1,12-Diacetate (9). To glaucarubinone triacetate (14, 200 mg, 0.323 mmol, prepared from 6 by the literature method[4]) in chloroform (4 ml) was added 1:9 water–methanol (4 ml) and triethylamine (0.5 ml). After 15 h at room temperature the sample was concentrated, chromatographed (ChromAR, 3% methanol–chloroform), and crystallized from acetone–hexane to give 9 as colorless plates (136 mg, 73%): mp 159–161°; $[\alpha]^{26}_D$ +16° (*c* 0.12, $CHCl_3$); uv max (EtOH) λ 239 nm (ε 10750); ir (KBr) 2.85, 5.72, 5.93, 7.25, 8.06, 13.3 μ; NMR ($CDCl_3$) τ 9.06 (3 H, d, *J* = 7 Hz, 13-CH_3), 9.04 (3 H, t, *J* = 7 Hz, $-CH_2CH_3$), 8.62 (3 H, s, 10-CH_3), 8.58 (3 H, s, 2'-CH_3), 8.11 (3 H, br s, 4-CH_3), 7.90, 7.83 (each 3 H, s, $-OCOCH_3$), 7.14 (1 H, s, 9-H), 6.33, 6.05 (each 1 H, d, *J* = 9 Hz, $-CH_2O-$), 5.42 (1 H, br s, 7-H), 5.08 (1 H, d, *J* = 4 Hz, 12-H), 4.74 (1 H, s, 1-H), 4.38 (1 H, d, *J* = 11 Hz, 15-H), 3.98 (1 H, br s, 3-H); mass spectrum *m/e* 578 (M^+), 536, 476, 420, 247, 151, 135, 73, 43. Anal. ($C_{29}H_{38}O_{12}\cdot H_2O$) C, H.

Glaucarubolone 15-Tiglate (10). To 9 (120 mg, 0.208 mmol) in pyridine (4 ml), cooled in an ice bath, was added dropwise thionyl chloride (0.5 ml). The mixture was kept at 0° for 5 min and then at room temperature for 40 min. Concentration and chromatography (ChromAR, 3% methanol–chloroform) gave a chromatographically homogeneous product (70 mg). This product was treated with 1% methanolic potassium hydroxide (8 ml) and the suspension was stirred at room temperature for 30 min. Acidification (hydrochloric acid in methanol), concentration, and chromatography (ChromAR, 3% methanol–chloroform) gave 10 (18 mg, 31%): $[\alpha]^{26}_D$ +65.4° (*c* 0.13, $CHCl_3$); uv max (EtOH) λ 238 nm (sh, ε 10250), 220 (14100); ir (KBr) 2.90, 3.03, 5.73, 5.96, 8.13, 8.80, 9.52, 13.4 μ; NMR ($CDCl_3$) τ 8.88 (3 H, d, *J* = 7 Hz, 13-CH_3), 8.79 (3 H, s, 10-CH_3), 8.18 (3 H, d, *J* = 4 Hz, $=CHCH_3$), 8.13 (3 H, s, 2'-CH_3), 7.97 (3 H, s, 4-CH_3), 7.24 (1 H, s, 9-H), 6.42 (1 H, d, *J* = 4 Hz, 12-H), 6.32, 6.02 (each 1 H, d, *J* = 9 Hz, $-CH_2O-$), 5.92 (1 H, s, 1-H), 5.31 (1 H, br s, 7-H), 4.45 (1 H, d, *J* = 11 Hz, 15-H), 3.85 (1 H, br s, 3-H), 3.04 (1 H, q, *J* = 4 Hz, $=CHCH_3$); mass spectrum *m/e* 476 (M^+), 458, 377, 376, 247, 151, 135, 83, 55. Anal. ($C_{25}H_{32}O_9\cdot 0.5H_2O$) C, H.

Glaucarubolone Tribenzoate (13) from Glaucarubolone (12). To a solution of 12 (69 mg, 0.175 mmol, obtained from 6 in 60% yield by the literature procedure[4]) in pyridine (2 ml) was added benzoyl chloride (0.5 ml). After standing overnight the mixture was concentrated, chromatographed (ChromAR, 1:4 ether–benzene), and crystallized from acetone–hexane to give 13 (121 mg, 97%): mp 163–167°; $[\alpha]^{26}_D$ +11° (*c* 0.23, $CHCl_3$); uv max (EtOH) λ 282 nm (ε 2800), 275 (3550), 233 (50500); ir (KBr) 2.86, 5.78, 5.93, 7.95, 9.01, 14.0 μ; NMR ($CDCl_3$) τ 8.72 (3 H, d, *J* = 7 Hz, 13-CH_3), 8.35 (3 H, s, 10-CH_3), 7.97 (3 H, s, 4-CH_3), 6.23 (1 H, s, 12-H), 5.94, 5.14 (each 1 H, d, *J* = 13 Hz, $-CH_2O-$), 5.82 (1 H, s, 9-H), 4.63 (1 H, br s, 7-H), 4.50 (1 H, s, 1-H), 3.87 (1 H, br s, 3-H), 3.73 (1 H, d, *J* = 9 Hz, 15-H), 2.49 (9 H, m, A_2B part of an A_2BX_2 system), 1.96 (6 H, m, X_2 part of an A_2BX_2 system); mass spectrum *m/e* 706 (M^+), 584, 462, 151, 149, 122, 105. Anal. ($C_{41}H_{38}O_{11}$) C, H.

Glaucarubolone 15-Benzoate (11). Treatment of 13 (100 mg, 0.142 mmol) in 1:9 water–methanol (10 ml) with triethylamine (1 ml) for 15 h at room temperature followed by concentration and chromatography (ChromAR, 3% methanol in chloroform) gave as the major component a colorless glass (49 mg) which, on crystallization from acetone–hexane, gave 11 as small needles (34 mg, 48%): mp 240–241°; $[\alpha]^{26}_D$ +74.2° (*c* 0.12, $CHCl_3$); uv max (EtOH) λ 282 nm (ε 680), 275 (990), 233 (20200); ir (KBr) 2.88, 3.03, 5.72, 5.78, 5.95, 8.13, 8.95, 13.8 μ; NMR ($CDCl_3$) τ 8.86 (3 H, d, *J* = 6.5 Hz, 13-CH_3), 8.77 (3 H, s, 10-CH_3), 7.94 (3 H, s, 4-CH_3), 7.16 (1 H, s, 9-H), 6.36 (1 H, d, *J* = 2 Hz, 12-H), 6.28, 5.99 (each 1 H, d, *J* = 9 Hz, $-CH_2O-$), 5.84 (1 H, s, 1-H), 5.26 (1 H, br s, 7-H), 4.20 (1 H, d, *J* = 11 Hz, 15-H), 3.83 (1 H, br s, 3-H), 2.51 (3 H, m, A_2B part of an A_2BX_2 system), 1.92 (2 H, dd, *J* = 6, 1 Hz, X_2 part of an A_2BX_2 system); mass spectrum *m/e* 498 (M^+), 480, 246, 151, 135, 122, 105. Anal. ($C_{27}H_{30}O_9$) C, H.

Dihydroglaucarubolone 15-Benzoate (15). A solution of 11 (180 mg, 0.362 mmol) in absolute ethanol (100 ml) was subjected to atmospheric hydrogenation at room temperature for 16 h using 5% palladium on charcoal (200 mg) as catalyst. Filtration, concentration, and crystallization from acetone–hexane gave 15 (150 mg, 83%): mp 233–235°; uv max (EtOH) λ 281 nm (ε 890), 274 (1040), 231 (13500); ir (KBr) 2.80, 3.11, 5.78, 8.13, 13.9 μ; NMR ($CDCl_3$) τ 9.10, 9.01, 8.91, 8.84, 8.75 (9 H, peak ratio 7:15:26:36:36,

respectively; 4-, 10-, and 13-CH_3 signals of the isomer mixture), 6.44 (1 H, d, J = 2 Hz, 12-H), 6.31, 6.03 (each 1 H, d, J = 9 Hz, -OCH_2-), 5.90 (1 H, br s, 1-H), 5.31 (1 H, br s, 7-H), 4.16 (1 H, d, J = 11 Hz, 15-H), 2.50 (3 H, m, A_2B part of an A_2BX_2 system), 1.91 (2 H, dd, J = 6, 1 Hz, X_2 part of an A_2BX_2 system); mass spectrum m/e 500 (M^+), 482, 379, 249, 105. Anal. ($C_{27}H_{32}O_9$) C, H.

On ChromAR **15** was clearly resolved into two components using ethyl acetate–cyclohexane (1:1) whereas on SilicAR best resolution was observed in 10% acetone–ether. Column chromatography (138 g, SilicAR CC-7, Mallinckrodt; 2.5 × 60 cm column) of the reduction product from 498 mg of **11** using anhydrous ether (Baker AR) as eluent gave an early fraction (62.3 mg) of the high R_f component (**15a**), and crystallization from acetone gave an analytical sample. Chromatography on ChromAR (17 plates; double development with 1:1 ethyl acetate–cyclohexane) of 21.4 mg of a column fraction containing approximately equal portions of **15a** and **15b**, followed by rechromatography of each of the components under identical conditions, gave 9.1 mg of **15a** and 8.7 mg of **15b**. The two components, which showed identical ir (KBr) and mass spectra, were easily differentiated by NMR: **15a** ($CDCl_3$) τ 8.99 (3 H, d, J = 7 Hz, 4-CH_3), 8.88 (3 H, d, J = 7 Hz, 13-CH_3), 8.76 (3 H, s, 10-CH_3); **15b** ($CDCl_3$) τ 8.91, 8.85, 8.75 (br, CH_3) and ($CDCl_3$ + C_6D_6) τ 9.21 (3 H, d, J = 5 Hz), 9.08 (3 H, s, 10-CH_3), 8.95 (3 H, d, J = 6.4 Hz).

Dihydroglaucarubinone (16). A solution of **6** (50 mg, 0.101 mmol) in absolute ethanol (50 ml) was subjected to atmospheric hydrogenation at room temperature for 16 h using 5% palladium on charcoal (50 mg) as catalyst. Filtration, concentration, and crystallization from acetone–hexane afforded **16** (34 mg, 67%) as small needles: mp 220–223°; uv (EtOH) λ 239 nm (ϵ 69); ir (KBr) 3.03, 5.75, 8.78, 9.54 μ; NMR ($CDCl_3$) τ 9.03 (3 H, t, J = 7 Hz, -CH_2CH_3), 8.91 (6 H, m, 4-CH_3, 13-CH_3), 8.77 (3 H, s, 10-CH_3), 8.55 (3 H, s, 2′-CH_3), 6.46 (1 H, br s, 12-H), 6.33, 6.08 (each 1 H, d, J = 9 Hz, -OCH_2-), 5.93 (1 H, br s, 1-H), 5.39 (1 H, br s, 7-H), 4.33 (1 H, d, J = 12 Hz, 15-H); mass spectrum m/e 496 (M^+), 478, 379, 250, 231, 73. Anal. ($C_{25}H_{36}O_{10}$) C, H.

Chromatography of a sample of **16** (68.5 mg) on ChromAR (21 plates developed three times with 3:2 ethyl acetate–cyclohexane) resolved the product into two bands which were each rechromatographed on ChromAR (20 plates developed three times with 3:1 ethyl acetate–cyclohexane) to give a higher R_f component (**16a**, 28.4 mg) and a lower R_f component (**16b**, 17.0 mg). Although the two products were indistinguishable by ir (KBr) and mass spectra, the NMR spectra (acetone-d_6) showed 10-CH_3 signals at τ 8.74 for the higher R_f component and at 8.85 for the lower R_f component.

References and Notes

(1) (a) Tumor Inhibitors. 115. For part 114, see S. M. Kupchan and A. Karim, *Lloydia*, in press. (b) The work was supported by a contract with the Division of Cancer Treatment, National Cancer Institute (N.C.I.), National Institutes of Health, Department of Health, Education and Welfare (NO1-CM-12099), research grants from the N.C.I. (CA-11718) and the American Cancer Society (CI-102J), and a Postdoctoral Fellowship (G.A.H., CA-02060-01) from the N.C.I.

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Phosphorus–Nitrogen Compounds. 20. Thiophosphorus Hydrazones

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Six pyridine-2-carboxaldehyde, one pyridine *N*-oxide 2-carboxaldehyde, and five diketone thiophosphoric hydrazones, three thiophosphoric hydrazides, and two cupric chelates were synthesized. The chelates and nine of the hydrazones were tested against Ehrlich ascites carcinoma. Seven of these latter agents were administered concurrently with either cupric and/or ferrous salts to mice bearing this tumor. The greatest activity was found with the chelate, dimethyl pyridine-2-carboxaldehyde phosphorothioic hydrazone–copper (1:1). The hydrazone portion of this chelate also formed a ligand–copper (2:1) complex. Although all of the hydrazones but one were inactive when evaluated alone, the concurrent injection of cupric ion increased survival times by an average of 145% T/C in four compounds so tested. *Escherichia coli* alkaline phosphatase was found to be inhibited by two thiosemicarbazones in a manner similar to that previously reported by these agents against alkaline phosphatase derived from Sarcoma 180 6-thiopurine resistant ascites cells. None of the 14 hydrazides or hydrazones tested against *E. coli* enzyme displayed significant inhibition.

Thiosemicarbazones and their metal chelates have been investigated as medicinal agents since Domagk[1] discovered antitubercular activity in this class of chemicals and, subsequently, they have been studied for antifungal, antiviral, and oncolytic properties.[2]

It is generally considered that these compounds function as ligands in chelation and their role in cancer chemotherapy has been recently reviewed.[3] In the past few years, heterocyclic carboxaldehyde and aliphatic diketone thiosemicarbazones and their metal chelates have been tested in animal model tumor systems, as well as ribonucleoside diphosphate reductase (RDR) and alkaline phosphatase (AP) inhibition studies.[2,4,5]

This paper reports the synthesis of phosphorus analogues of pyridine-2-carboxaldehyde, one *N*-oxide analogue, and aliphatic diketone thiosemicarbazones and their effects as inhibitors of the action of *Escherichia coli* AP and the growth of Ehrlich ascites carcinoma cells in mice. Selected agents were tested by this latter method as cupric complexes and via concurrent administration of cupric and ferrous ions.

Chemistry. Three previously unreported thio-