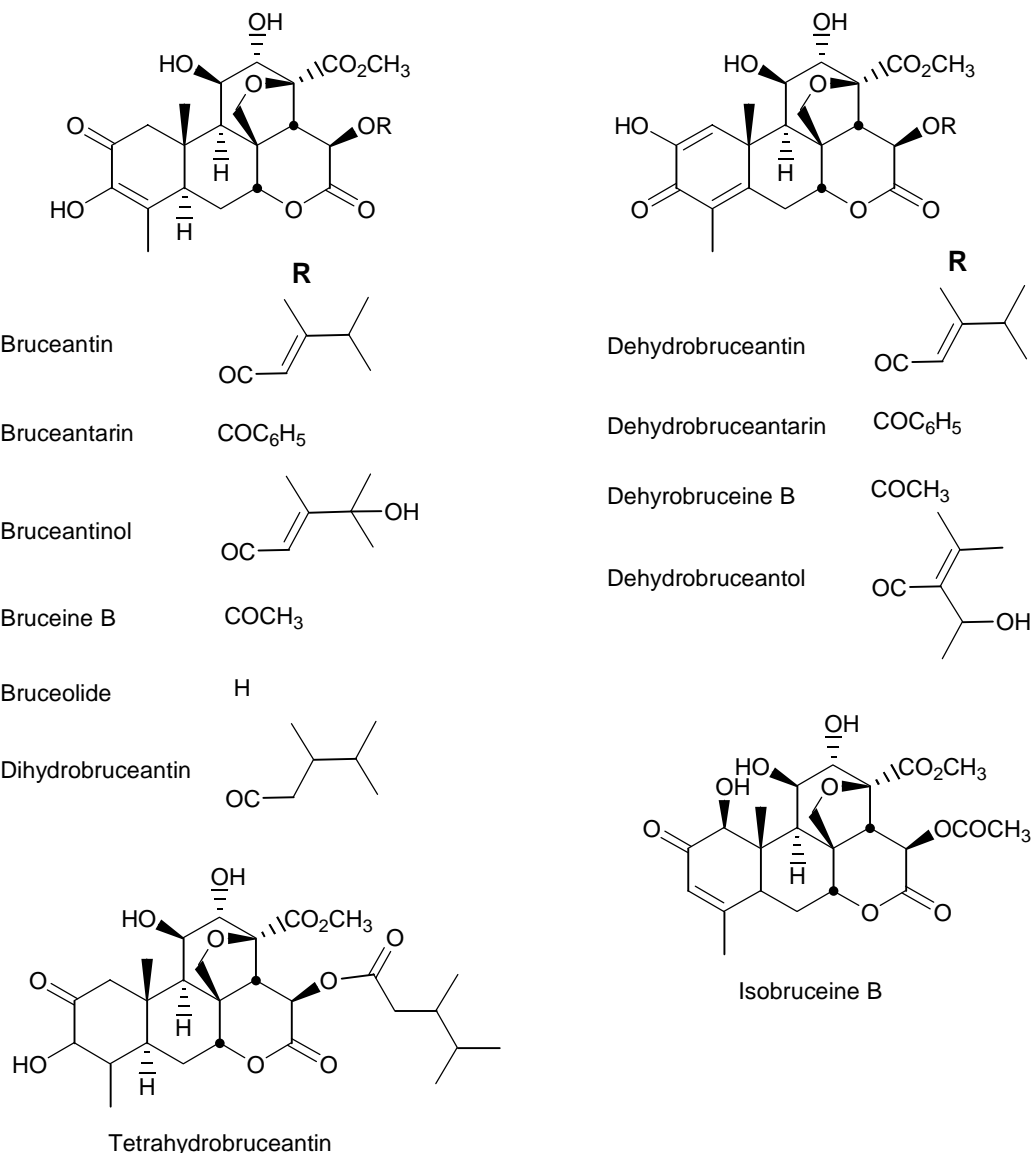


## Bruceantin and Its Homologues

Bruceantin is a triterpene of the quassinoid type isolated by Kupchan and co-workers from the bark of the Ethiopian tree *Brucea antidysenterica* Mill. (Simaroubaceae) in 1972.<sup>1</sup> Fractionation of the ethanolic extract of the bark was guided by bioassays against the P388 lymphocytic leukemia in mice and the 9KB cell culture derived from a human epidermoid carcinoma. Nine additional quassinoids, bruceantarin, bruceantanol, bruceine B, bruceolide, dehydrobruceantin, dehydrobruceantarin, dehydrobruceine B, dehydrobruceantol, and isobruceine B were subsequently isolated from the same plant.<sup>2</sup> Bruceantin, bruceantarin, bruceantanol, bruceine B, and dehydrobruceantin were also isolated from the Ghanaian tree *Brucea guineensis* G. Don.<sup>2</sup>



Bruceantin demonstrated significant activity *in vivo* against several tumor systems (Table 1) including the P388 lymphocytic leukemia (PS), the L1210 lymphoid leukemia (LE), an adriamycin resistant P388 leukemia (PA), a cytoxan resistant P388 leukemia (PO), the B16 melanocarcinoma (B1), and the Lewis

Lung carcinoma (LL).<sup>3</sup> The wide dose range and relatively low doses required for activity elicited significant interest in bruceantin as a potential antineoplastic agent. Bruceantin also inhibited, irreversibly, protein synthesis in HeLa cells, rabbit reticulocytes, and reticulocyte lysates, and partially inhibited DNA synthesis in HeLa cells, but had no effect on RNA synthesis.<sup>4</sup>

TABLE 1 Activity (T/C\*) of Bruceantin Against In Vivo Tumor Systems

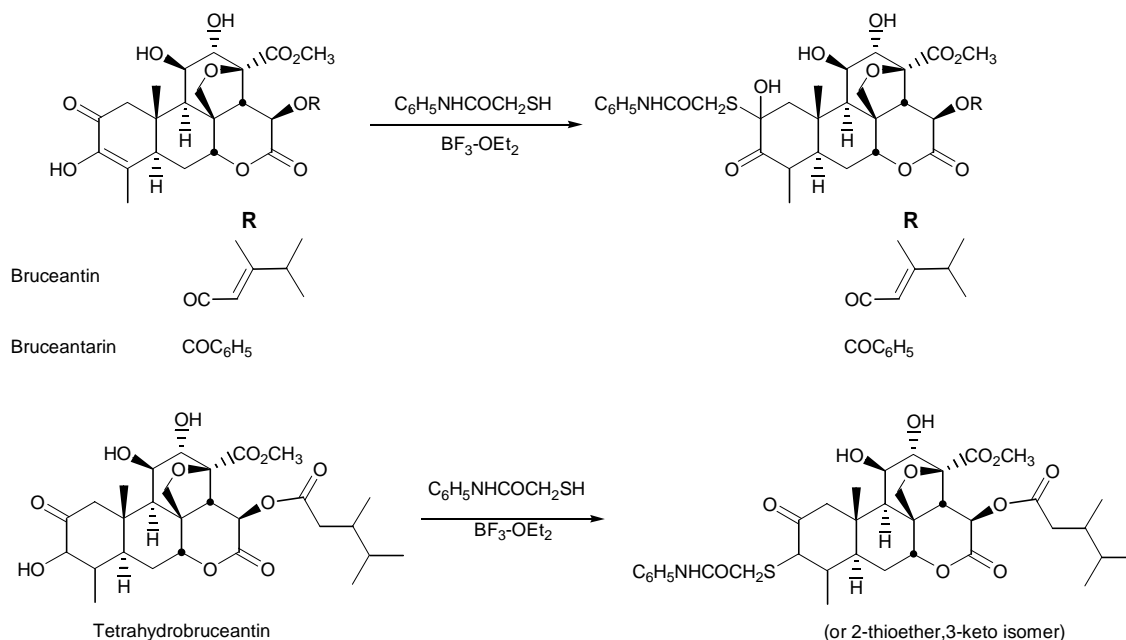
Dose (mg/kg)	Tumor Systems					
	PS	LE	B1	LL	PA	PO
2.25					149	
1.50					145	
1.00	140	141	164	133	155	198
0.66					149	205
0.50	220	135	168	128		
0.44					125	209
0.25	195	128	160	128		
0.125	135	127	160			
0.062	165		145			
0.031	150					

Several of the homologues of bruceantin also exhibited significant activity in the P388 and 9KB systems. The most potent of these, however, was bruceantinol (Table 2), suggesting the requirement of an  $\alpha,\beta$ -unsaturated C-15 ester for optimal activity.<sup>5</sup> Dihydrobruceantin, a semisynthetic homologue in which the double bond of the C-15 ester has been hydrogenated, showed diminished activity, and bruceantarin, bruceine B, and bruceolide showed only marginal activity. One role of the C-15 ester is likely to be transport of the compound across cell membranes. Inhibition of protein synthesis in HeLa cells and rabbit reticulocytes paralleled the relative order of antileukemic activity. Bruceantin and dihydrobruceantin were the most active ( $IC_{50}$   $10^{-8}$  M). Bruceantarin and bruceine B were approximately 10X less active, and bruceolide was about 100X less active. All five compounds, however, were essentially equal in activity in reticulocyte lysates, where transport through a cell membrane is not a factor.

TABLE 2 Antileukemic Activity of Quassinoids from *Brucea Antidysenterica* Against the P-388 Lymphocytic Leukemia In Vivo (T/C)

Compound	Dose (mg/kg)							
	4.0	2.0	1.0	0.5	0.25	0.125	0.06	0.03
Bruceantin	Toxic	Toxic	140	220	195	135	165	150
Bruceantarin		150	145	135	125	135	140	100
Bruceantinol		194	238	211	200	183	183	133
Bruceine B			130	115	110	105	100	100
Bruceolide	147	110	137	117	99			
Dehydrobruceantin		110	120	105	100	135	115	120
Dehydrobruceantarin	100			133*				111**
Dihydrobruceantin	163	168	163	140	136	136	122	113
Tetrahydrobruceantin	126	108	103	103	106	115	105	

Tetrahydrobruceantin was inactive at doses equivalent to active doses for bruceantin, and the dehydro-homologues were essentially inactive, as well. This indicated that the A-ring diosphenol moiety was directly involved in the reactions which result in tumor-inhibitory activity. These reactions are likely to be alkylation of biological sulfhydryl or amine moieties, and this was demonstrated in the case of bruceantin by the reaction of thioglycolic acid anilide with selected bruceolides.<sup>6</sup> With bruceantarin and dihydrobruceantin, the product of the reaction was a C-2 hemithioketal. However, with tetrahydrobruceantin, the product was a substitution product. This difference in chemical reactivity suggests a difference in biological reactivity which may be related to the differences in biological activity.



Due to its significant activity in several animal tumor systems, bruceantin was selected for advancement to clinical trials by the NCI. Preclinical toxicology studies in dogs and monkeys indicated cardiovascular toxicity, edema, diarrhea, emesis, erythema, and some hemorrhaging.<sup>7</sup> In dogs, single doses of  $>10$   $mg/m^2$  caused death in 12-16 hours. However, single doses of  $<10$   $mg/m^2$  or daily dose regimens of 2.5  $mg/m^2/day$  gave reversible toxic side effects. Extended dose regimens with resting periods in between indicated that toxicity was seen only during the treatment period. Similar experiments in monkeys gave similar results, suggesting that the toxicity of bruceantin was treatable and not cumulative. Bruceantin began Phase I clinical trials in the fall of 1977. Some promise was shown against selected cancers, and Phase II trials were recommended. However, adequate quantities of the compound became more difficult to obtain because of the location of the primary source, *B. antidysenterica*, and, combined with the advent of more promising drug candidates, this resulted in it being dropped from consideration in the early 1980's.

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