Antitumor Agents LIX: Effects of Quassinoids on Protein Synthesis of a Number of Murine Tumors and Normal Cells

I. H. HALL *x, Y. F. LIOU *, K. H. LEE *, S. G. CHANEY ‡, and W. WILLINGHAM, JR. ‡

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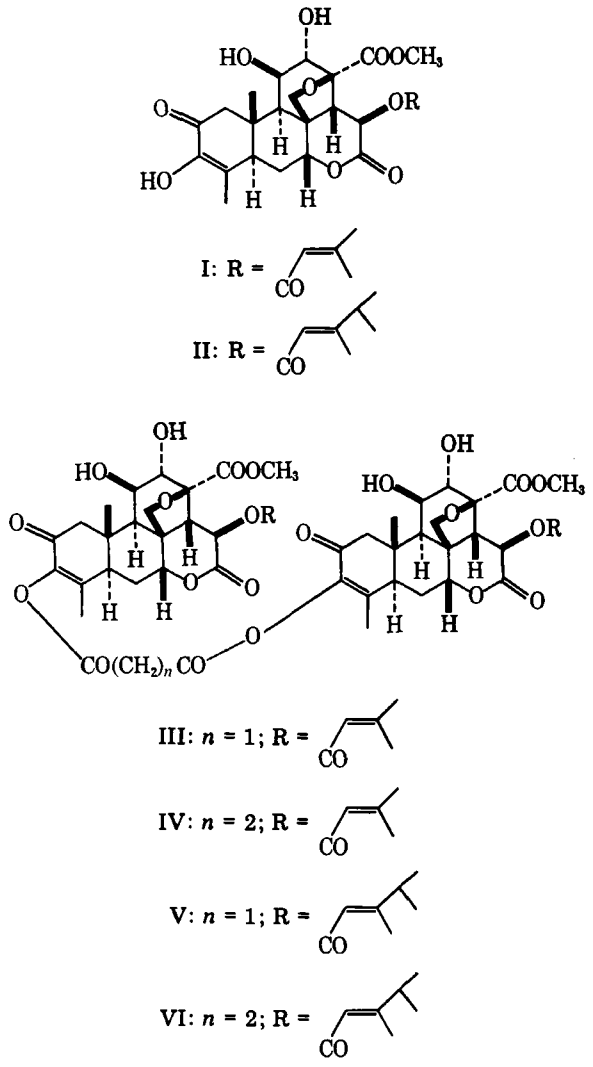
Abstract □ The quassinoids (brusatol, bruceantin, bisbrusatolyl esters, and bisbruceantinyl esters of succinic and malonic acids) were observed not to be universal protein synthesis inhibitors. Rather, they were selective for both the types of cancers, *e.g.*, P-388 lymphocytic leukemia, Ehrlich and hepatoma carcinoma and L-1210 lymphoid leukemia, as well as types of normal tissues (*e.g.*, lymphocytes), in which they demonstrated protein synthesis inhibition. The data suggest that the observed difference in the magnitude of protein synthesis inhibition of two P-388 lymphocytic leukemia cell lines by the quassinoids was at the ribosomal levels, whereas the observed difference in normal livers from various strains of mice involve differences in cell membrane transport of the quassinoids into the various tissues. Detailed studies indicated that the mode of action of the quassinoids as protein synthesis inhibitors was identical in all of the cells where inhibition was observed; *i.e.*, the elongation step of protein synthesis was blocked by the quassinoids. The data derived from assays for polyuridine-directed polyphenylalanine synthesis of isolated ribosomes demonstrated that the ID_{50} values obtained were consistent with the observed inhibition of whole cell protein synthesis inhibition for P-388 cells, as well as for BDF_1 and DBA/2 liver cells. The ID_{50} values obtained from various cells, *e.g.*, P-388 cells and normal liver, were all in the μM range and are consistent with previously published values for the quassinoids in the rabbit reticulocyte and yeast systems.

Keyphrases □ Brusatol—effects on protein synthesis of murine tumors □ Bruceantin—effects on protein synthesis of murine tumors □ Bisbrusatolyl esters—effects on protein synthesis of murine tumors □ Protein synthesis—inhibition by quassinoids, P-388 lymphocytic leukemia cells

Recently a number of quassinoids including brusatol (I), bruceantin (II), bisbrusatolyl malonate (III), bisbrusatolyl succinate (IV), bisbruceantinyl malonate (V), and bisbruceantinyl succinate (VI) were observed to inhibit *in vitro* protein synthesis of P-388 lymphocytic leukemia cells (1). The inhibition of protein synthesis correlated positively with P-388 tumor cell growth inhibition (2), and further work demonstrated that the elongation step of protein synthesis was being inhibited by the quassinoids (1–3). In earlier studies, this laboratory observed that different lines of P-388 lymphocytic cells, P-388-UNC and P-388-NIH, metabolically responded quantitatively differently to the quassinoids; *i.e.*, in the two cell lines different magnitudes of protein synthesis inhibition were observed. Thus, the objectives of the present study were to examine (*a*) the effects of time of incubation to determine how rapidly the agents act on protein synthesis, (*b*) the effects of quassinoids on protein synthesis in two lines of P-388 cells as well as other murine tumor lines, (*c*) the effects of quassinoids on normal tissue protein synthesis to determine if they are ubiquitous mammalian protein synthesis inhibitors, and (*d*) the effects of the quassinoids on *in vivo* protein synthesis using multiple doses and different concentrations (mg/kg) of drugs.

EXPERIMENTAL

Sources of Compounds—The sources of the quassinoids as well as their physical and chemical characteristics have previously been reported



Structures of Quassinoids

(4, 5). All of the agents were homogenized in 0.05% polysorbate-80–water for use in the *in vivo* and *in vitro* studies.

Sources of Tumor and Normal Cells—P-388 lymphocytic leukemia cells were obtained through the National Cancer Institute. The P-388-UNC tumor line was obtained in June 1978 and the P-388-NCI was obtained in December 1980. Both P-388 lymphocytic leukemia lines were maintained in DBA/2 or BDF_1 male mice (~18 g). On day zero, 10^6 P-388 cells were implanted intraperitoneally, and on day 9 the cells were harvested for the *in vitro* studies. Antineoplastic activity was determined for P-388 screen by the NCI protocol (6). The L-1210 lymphoid leukemia line was maintained in DBA/2 male mice (~20 g). On day zero, 10^5 cells were implanted intraperitoneally, and the cells were harvested on day 8 for *in vitro* studies. The hepatoma 129 line was maintained similarly to the P-388 line except the host species was male C_3H mice (~18 g). Cells for the *in vitro* study were removed on day 8. The renal cell carcinoma (RCO_2) was maintained in CDF male mice (~20 g). On day zero, 0.1 ml of ascites fluid was implanted into new donor mice. Three weeks later, cells were harvested for *in vitro* studies.

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Table I—The Effect of Quassinoid Esters on Protein Synthesis of Two Strains of P-388 Lymphocytic Leukemia Cells for 90-min Incubation

	Control, %									
	P-388-UNC					P-388-NCI				
	5 μM	10 μM	15 μM	25 μM	50 μM	5 μM	10 μM	15 μM	25 μM	50 μM
0.05% Polysorbate 80	100 ± 7	100 ± 7	100 ± 7	100 ± 7	100 ± 7	100 ± 8	100 ± 8	100 ± 8	100 ± 8	100 ± 8
Brusatol	23 ± 6	22 ± 4	19 ± 4	18 ± 3	16 ± 3	24 ± 7	23 ± 5	20 ± 6	18 ± 5	13 ± 3
Bruceantin	75 ± 7	57 ± 6	49 ± 7	38 ± 6	32 ± 4	28 ± 4	19 ± 4	18 ± 6	17 ± 3	19 ± 3
Bisbrusatolyl malonate	54 ± 5	17 ± 4	14 ± 3	5 ± 2	5 ± 2	85 ± 7	70 ± 6	55 ± 5	50 ± 7	50 ± 7
Bisbrusatolyl succinate	58 ± 4	28 ± 4	23 ± 3	—	—	87 ± 9	75 ± 5	63 ± 6	—	—
Bisbruceantinyl malonate	72 ± 5	60 ± 6	45 ± 4	—	—	81 ± 7	76 ± 6	68 ± 6	—	—
Bisbruceantinyl succinate	78 ± 6	68 ± 5	50 ± 4	—	—	90 ± 8	78 ± 6	70 ± 7	—	—

All of the solid tumors were maintained in male $C_{57}B1/6$ mice (~22 g). On day zero a fragment (~30 mg) was implanted into the mice. For the B-16 melanotic melanoma, brain glioma 261 and the ependymoblastoma, the fragment was implanted into the inguinal region of the hind leg. For the Lewis lung, the tumor was implanted intramuscularly into the hind leg. Cells were harvested on day 14 and homogenized with a loose pestle to obtain individual cells in 0.25 *M* sucrose and 0.001 *M* EDTA (ethylenediaminetetraacetic acid), pH 7.4.

The Ehrlich ascites tumor line was maintained in CF_1 male mice (~20 g) by implanting 2×10^6 cells on day zero. Cells were collected on day 8. The sarcoma 180 ascites tumor line was maintained in Swiss Webster male mice (~20 g). Cells were harvested on day 8. The KB human epidermoid carcinoma of the mouth was maintained as a tissue culture in *minimum essential medium and 10% fetal calf serum containing the* antibiotics, penicillin and streptomycin (7).

Normal tissues were selected from the strain of mice that acted as host for the tumor transplant. Normal kidneys were obtained from CDF_1 male mice; normal brain tissue was obtained from $C_{56}B1/6$ male mice; normal liver was obtained from C_3H male mice, and spleen lymphocytes were obtained from DBA/2 male mice.

***In Vitro* Whole Cell Leucine Incorporation into Protein**—These studies were conducted with 10^6 cells (homogenized and whole cell) from tumor or normal tissues, 1 μCi of L-leucine [4,5-^{3}H, 56.5 Ci/mmole] with minimum essential medium in a total volume of 1 ml, which was incubated 60 min at 37°. The time of incubation was varied in the P-388, L-1210, hepatoma, and Ehrlich ascites studies from 30–120 min. The reaction was stopped with 10% trichloroacetic acid, and the insoluble protein was collected on filters[1] by vacuum suction and the filters counted[2] (8, 9). The results are expressed as dpm/10^6 cells.

Polyuridine-Directed Polyphenylalanine Synthesis—Tumor lysates were prepared by a previous method (10). The following were isolated from tumor lysates by literature techniques: runoff ribosomes (11), pH 5 enzymes (10), and uncharged tRNA (12). The reaction medium (13) contained 50 m*M* *tris*(hydroxymethyl)aminomethane, pH 7.6, 12.5 m*M* magnesium acetate, 80 m*M* KCl, 5 m*M* creatine phosphate, 0.05 mg/ml creatine phosphokinase, 0.36 mg/ml polyuridine [poly(U)]-(A_{280}/A_{260} = 0.34), 0.5 μCi [^{3}H]phenylalanine (536 mCi/mmole), 75 μg uncharged tumor cell tRNA, 70 μg of tumor pH 5.0 enzyme preparation, and 0.9 A_{260} of P-388 tumor cell or liver runoff ribosomes. Test drugs were present in 0–30 μ*M* concentration. Incubation was for 20 min at 30° after which a 35-μl aliquot was spotted on filter papers[3] which were treated for 10 min in boiling 5% trichloroacetic acid and washed with cold 5% trichloroacetic acid, ether–ethanol (1:1), and ether. The filter papers were dried and counted in scintillation fluid[2].

***In Vivo* Studies of Protein Synthesis Inhibition**—In order to determine the effects of quassinoids on normal *in vivo* protein synthesis, CF_1 male mice (~22 g) were injected with brusatol, bruceantin, or bisbrusatolyl malonate at a dose of 0.6 mg/kg ip for 3 days. On the 4th day, 1 hr prior to sacrifice, 10 μCi of [^{3}H]leucine was injected intraperitoneally. The liver, kidney, spleen, and lung were excised, and the protein was extracted by a previous method (14) and counted. Protein content was determined by another method (15). *In vivo* P-388 tumor cell studies were conducted to determine the dose response effect on protein synthesis by implanting 10^6 cells on day zero into BDF_1 mice. On days 7, 8, and 9, brusatol, bruceantin, or bisbrusatolyl malonate was injected intraperitoneally from 0.10 to 0.60 mg/kg/day. On day 10, the cells were harvested and extracted as described above. In other studies, brusatol and bruceantin were injected as a single dose on day 7, as two doses on days 7 and 8, and three doses on days 7, 8, and 9 at 0.3 mg/kg/day. Cells were harvested 6 hr after the last dose. Protein was extracted (14), counted, and protein content (15) determined. Results are expressed as dpm/mg of isolated protein.

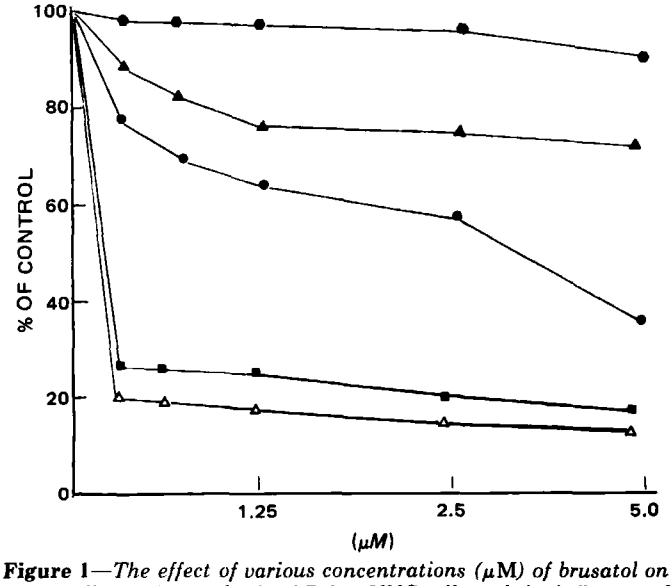

Figure 1—*The effect of various concentrations (μM) of brusatol on whole cell protein synthesis of P-388-UNC cells and the influence of time of incubation. Key: (●) 30 min; (▲) 45 min; (●) 60 min; (■) 90 min; (△) 120 min.*

RESULTS

In initial studies, brusatol (I), bruceantin (II), and bisbrusatolyl malonate (III) were incubated with P-388-UNC lymphocytic leukemia cells between 30–120 min. Brusatol required more than 60 min incubation to obtain maximal inhibition (Fig. 1). Little difference was observed in the extent of inhibition after 90- or 120-min incubations. Similar data were obtained with bruceantin and bisbrusatolyl malonate. Thus, 90-min incubations were used routinely in subsequent experiments. Under these conditions, brusatol was the most effective inhibitor causing an inhibition of 75–80% at concentrations between 1.25 and 5 μ*M* (Fig. 1). The quassinoid esters, III and IV, caused the same magnitude of protein synthesis inhibition but required concentrations of 10–15 μ*M* (Table I). Compound II was not that active as a protein synthesis inhibitor at this concentration range in P-388 cells (Table I). Concentrations above 10 μ*M* were required for II to afford more than 50% inhibition of protein synthesis.

Comparison between two P-388 lymphocytic leukemia lines of tumor (P-388-UNC and P-388-NCI) shows that the quassinoids have a differential effect on tumor growth suppression as well as protein synthesis inhibition (Table I). Compounds I and II at 0.6 mg/kg/day were slightly more active against the growth of the P-388 NCI tumor line compared with the P-388-UNC line (Table II). Compound III was less active against the growth of the P-388-NCI tumor line compared with the P-388-UNC tumor line. Protein synthesis was inhibited (Table II) in a similar manner; *e.g.*, I and II were slightly better inhibitors in the P-388-NCI tumor cell system, whereas III had less effect on protein synthesis in the P-388-NCI tumor line. Approximately the same degree of inhibition of protein

[1] Millipore nitrocellulose.

[2] Fisher Scintiverse in a Packard Counter.

[3] Whatman No. 3.

Table II—The Effects of Quassinoids on *In Vivo* P-388 Lymphocytic Leukemia Tumor Growth

		T/C% at 0.6 mg/kg/day	
		P-388 UNC	P-388 NCI
I	Brusatol	149	156
II	Bruceantin	146	162
III	Bisbrusatolyl malonate	212	135
IV	Bisbrusatolyl succinate	217	161
V	Bisbruceantinyl malonate	139	139
VI	Bisbruceantinyl succinate	153	151
	5 Fluorouracil (12.5 mg/kg)	186	209

synthesis was observed for homogenized cell preparations from both P-388 lines (data not shown). Since III is a dimer connected through an ester group, the question arose whether the difference in response in both antineoplastic activity and inhibition of protein synthesis could be the property of the ester linkage. Thus, three other esters were examined: bisbrusatolyl succinate (IV), bisbruceantinyl malonate (V), and bisbruceantinyl succinate (VI). As can be seen in Table II, all three of these esters demonstrated less antineoplastic activity in the P-388-NCI tumor line compared with the P-388-UNC tumor line. The same analogy can be observed with respect to protein synthesis inhibition; i.e., the esters had less activity as protein synthesis inhibitors in the P-388-NCI tumor cell line (Table II).

In vivo protein synthesis studies in BDF_1 mice inoculated with tumor P-388-NCI lymphocytic leukemia cells demonstrated that after a 3-day administration of the quassinoids from 0.10 to 0.60 mg/kg, brusatol had the strongest effect on protein synthesis inhibition causing greater than 80% inhibition at 0.10 mg/kg/day (Fig. 2). Compound II required a dose of 0.30 mg/kg to produce greater than 80% inhibition, whereas bisbrusatolyl malonate only caused 70% inhibition at 0.60 mg/kg for 3 days. Examination of single and multiple dosing of I, II, and III at 0.3 mg/kg shows that 6 hr after the first dose I caused >90% inhibition of *in vivo* protein synthesis (Fig. 3). Bruceantin, on the other hand, only caused 25% inhibition of protein synthesis 6 hr after a single dose, two doses caused 77% inhibition, and three doses were required to cause >90% inhibition of protein synthesis. Three doses of bisbrusatolyl malonate were required to cause greater than 50% inhibition of protein synthesis. Thus, the *in vivo* studies on the effects of quassinoids on P-388-NCI leukemia cell protein synthesis afforded consistent results with *in vitro* studies.

The quassinoids are potent inhibitors of the polyuridine-directed polyphenylalanine synthesis reaction of P-388 ribosomes. The ID_{50} values obtained for I, II, and III were for the P-388-UNC ribosomes 6.4, 11.5, and 1.9, respectively, and for the P-388-NCI ribosomes 0.4, 0.6, and 25 μM, respectively.

Since the specificity of the quassinoids as protein synthesis inhibitors varied so strikingly between the two P-388 cell lines, the effects of these compounds on the inhibition of *in vitro* protein synthesis of a series of unrelated murine tumors were also examined. As can be seen in Table III, compounds I, II, and III were found to be potent protein synthesis inhibitors in both Ehrlich and hepatoma 129 carcinomas at various concentrations. Again, 90-min incubations resulted in the maximum degree of protein synthesis by the quassinoids for inhibition in Ehrlich and hepatoma cells (data not shown). A moderate protein synthesis inhibition was observed by the quassinoids in the L-1210 lymphoid leukemia, B-16 melanotic melanoma, and ependymoblastoma tumor cells. Compounds I and II at the high concentrations (50 μM) produced mild inhibition of protein synthesis in the brain glioma 261 tumor cells. The quassinoids actually stimulated whole cell protein synthesis in the Lewis lung carcinoma, renal cell carcinoma, and human KB tissue culture system.

As can be observed from Table III, normal lungs from C_3H mice and normal kidneys from CDF mice demonstrated the same pattern of protein synthesis as the Lewis lung carcinoma and renal cell carcinoma of that tissue, *i.e.*, an increase in protein synthesis after 90-min incubation with the quassinoids. The quassinoids inhibited both normal spleen lymphocytic and lymphoid leukemia cells in DBA/2 mice. However, the quassinoids did not cause protein synthesis inhibition of normal liver from C_3H mice but did cause inhibition of the hepatoma from this same strain of mice.

As can be noted in Table III, only protein synthesis from noncancerous livers of BDF_1 and DBA/2 mice was inhibited by the quassinoids at 50-μM concentrations. All other whole cell liver studies from different strains of mice, CF_1, CDF, and $C_{56}B1/6$, demonstrated no inhibition of protein synthesis by the quassinoids. Since liver cells from most strains of mice seemed to be relatively resistant to inhibition by the quassinoids, the effect of these compounds on polyuridine-directed polyphenylalanine synthesis *in vitro* for two of these cell types was determined. In BDF_1 liver, compounds I, II, and III afforded ID_{50} values of 0.3, 0.6, and 25 μM, whereas the DBA/2 liver ribosomes, the ID_{50} values were 0.4, 0.7, and >50 μM, respectively. Bruceantin afforded an ID_{50} of >50 μM with CF_1 liver ribosomes.

DISCUSSION

The quassinoids do not appear to be universal protein synthesis inhibitors. Instead, they are selective for the tissue type as well as noncancerous and cancerous tissue cell types. In some cases this selectivity appears to be due to inherent differences at the ribosome level, while in other cases it seems to be a result of differences in tissue cell permeability to the quassinoids. In whole cell studies the quassinoids require an interval of time before they effectively bring about protein synthesis inhibition. This phenomenon may be due to delayed transport of the quassinoid into the cell, to a time lag necessary for binding of the quassinoids to their intracellular target, or to metabolism of the quassinoids to an active species which inhibits protein synthesis.

The quassinoids did appear to exhibit a quantitative difference in blocking protein synthesis of the P-388-NCI and P-388-UNC lymphocytic leukemia lines. The bis-quassinoid esters were not as active in the P-388-NCI tumor line compared with the P-388-UNC leukemia line. In the *in vivo* studies, the inhibition of protein by compounds I, II, and III after 3 days administration was dose related. Brusatol was more active than bruceantin, and bisbrusatolyl malonate was the least active in the

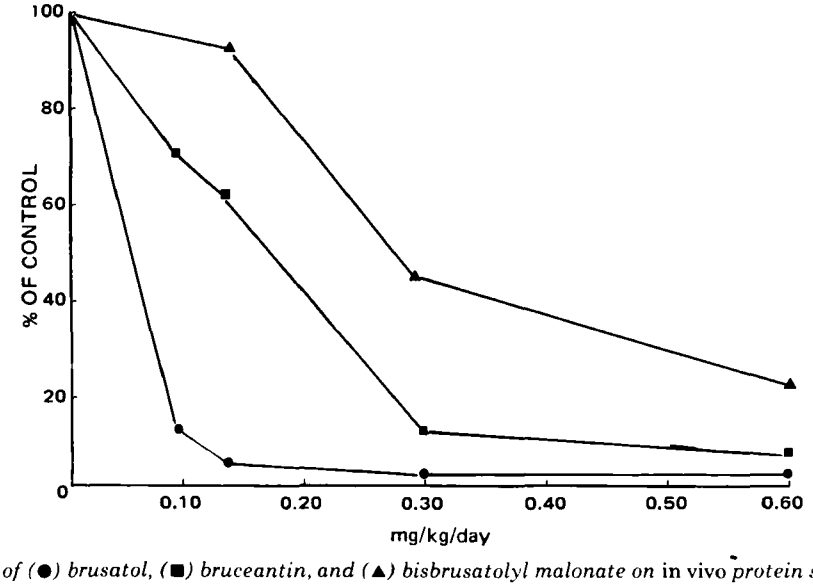

Figure 2—*The effect of dose of (●) brusatol, (■) bruceantin, and (▲) bisbrusatolyl malonate on* in vivo *protein synthesis of P-388-NCI cells.*

Table III—The Effects of Brusatol, Bruceantin, and Bisbrusatolyl Malonate on *In Vitro* Protein Synthesis on Normal and Carcinoma Tissues From the Same Species of Mice [a]

Cancerous Tissues	Control	Control, % Brusatol 10	Brusatol 25	Brusatol 50	Bruceantin 10	Bruceantin 25	Bruceantin 50	Bisbrusatolyl Malonate 10	Bisbrusatolyl Malonate 25	Bisbrusatolyl Malonate 50
Ehrlich Ascites Carcinoma in CF_1	100 ± 5	19 ± 3	23 ± 4	23 ± 3	15 ± 2	22 ± 3	20 ± 3	25 ± 4	27 ± 3	28 ± 3
Hepatoma from C_3H	100 ± 8	74 ± 6	76 ± 6	66 ± 4	78 ± 7	79 ± 6	76 ± 5	72 ± 6	78 ± 7	79 ± 7
L-1210 Lymphoid Leukemia in DBA/2	100 ± 10	72 ± 5	69 ± 4	71 ± 6	78 ± 7	79 ± 8	76 ± 5	72 ± 6	78 ± 7	79 ± 6
B-16 Melanotic Melanoma in $C_{57}B1/6$	100 ± 9	71 ± 6	64 ± 6	60 ± 7	79 ± 5	58 ± 4	52 ± 3	85 ± 6	78 ± 7	76 ± 7
Ependymoblastoma in $C_{57}B1/6$	100 ± 8	89 ± 8	79 ± 9	60 ± 6	84 ± 6	81 ± 5	76 ± 6	130 ± 9	81 ± 8	78 ± 8
Glioma 261 in $C_{57}B1/6$	100 ± 5	104 ± 7	62 ± 6	58 ± 7	112 ± 7	100 ± 6	76 ± 5	102 ± 6	103 ± 7	110 ± 8
Sarcoma 180	100 ± 6	102 ± 8	64 ± 7	50 ± 6	110 ± 7	88 ± 4	66 ± 7	122 ± 8	107 ± 8	81 ± 9
Lewis Lung Carcinoma from $C_{57}B1/6$	100 ± 7	137 ± 8	105 ± 6	123 ± 7	133 ± 8	132 ± 9	108 ± 7	111 ± 6	106 ± 5	134 ± 16
Renal Cell Carcinoma from CDF	100 ± 8	92 ± 7	387 ± 40	499 ± 35	94 ± 6	130 ± 7	208 ± 13	131 ± 10	243 ± 15	380 ± 16
Normal Tissue		12	25	50	12	25	50	12	25	50
Liver from C_3H	100 ± 9	133 ± 12	139 ± 13	142 ± 15	76 ± 6	94 ± 9	165 ± 12	122 ± 10	122 ± 12	129 ± 9
Liver from DBA/2	100 ± 7	92 ± 6	84 ± 6	44 ± 5	94 ± 6	75 ± 3	49 ± 4	79 ± 7	74 ± 5	79 ± 8
Liver from BDF	100 ± 6	83 ± 7	43 ± 5	22 ± 4	82 ± 4	61 ± 5	31 ± 4	72 ± 6	71 ± 7	59 ± 6
Lung from $C_{57}B1/6$	100 ± 7	137 ± 8	105 ± 6	123 ± 7	133 ± 8	132 ± 9	108 ± 7	111 ± 6	106 ± 5	134 ± 16
Kidney from CDF	100 ± 7	116 ± 8	110 ± 9	122 ± 11	106 ± 6	127 ± 12	127 ± 8	107 ± 6	129 ± 12	145 ± 20
Spleen Lymphocytes from DBA/2	100 ± 6	94 ± 6	96 ± 7	92 ± 5	80 ± 6	74 ± 6	63 ± 5	83 ± 5	76 ± 6	65 ± 4

[a] μM Concentrations.

P-388-NCI tumor line. The same relationship can also be observed among the quassinoids with respect to the time after administration of the agents to obtain significant protein synthesis inhibition. These quantitative differences were positively correlated with the antineoplastic activity of the quassinoids obtained in the individual P-388 tumor lines. However, in both P-388 tumor lines, the quassinoids acted identically as elongation inhibitors in polyuridine-directed polyphenylalanine synthesis in the fractionated system. Previous studies have shown that inhibition of the polyuridine-directed polyphenylalanine synthesis is a measure of their effectiveness as antineoplastic agents (2). This system does not require initiation or termination factors. Thus, an inhibitor of this reaction can be considered to be an elongation inhibitor of protein synthesis. Detailed studies have previously demonstrated that the peptidyl transferase reaction is blocked by the quassinoids (1–3). The ID_{50} values obtained for each of the quassinoids in the two P-388 cell lines were consistent with the observed whole cell protein synthesis inhibition; *i.e.*, I and II produced ID_{50} values of lower magnitude in the P-388-NCI line, whereas III afforded an ID_{50} value of lower magnitude in the P-388-UNC line. The fact that these quassinoids were equally effective in whole cell suspensions and in homogenates as well as fractionated protein-synthesizing systems derived from P-388 cells rules out differences in transport or metabolism of these drugs by the two different cell lines. In this case the quantitative differences between the sensitivities of these two cell lines appear to reside at the ribosome level.

The quassinoids were potent protein synthesis inhibitors of some murine tumors: Ehrlich ascites and hepatoma carcinoma were significantly inhibited, whereas L-1210 lymphoid leukemia, B-16 melanotic melanoma, and brain ependymoblastoma were moderately inhibited. However, the quassinoids had no effect on other tumors including human KB cells. A reason for this observation is not obvious, but may involve the transport or metabolism of the quassinoids at different rates or manners by the individual tumors or intrinsic differences in ribosome sensitivity to the drugs. In those tumors where whole cell protein synthesis was inhibited (Ehrlich ascites, hepatoma, and L-1210 lymphoid leukemia), similar times were required to observe maximum protein synthesis inhibition (90 min) as were observed for P-388 cells. There did not appear to be a selectivity of protein synthesis inhibition between normal and cancer cells in the same mouse species. Except for hepatoma in C_3H mice, the quassinoids were not specific inhibitors in cancer cells exclusively, but inhibited protein synthesis of some normal tissues such as spleen lymphocytes, which were proliferating regularly, and DBA/2 and BDF_1 liver cells. Interestingly, the ID_{50} values obtained in the polyuridine-directed polyphenylalanine system of ribosomes isolated from different strains of mice again shows that in those livers where good inhibition by the quassinoids of whole cell protein synthesis was observed, the ID_{50} values were of a low magnitude (0.4 and 0.6 μM), whereas when the whole cell inhibition was not significant, the ID_{50} values were high (>50 μM in CF_1 liver). The ID_{50} value for inhibition of polyphenylalanine synthesis in fractionated systems appeared to be in the same concentration range for both DBA/2 and BDF_1 liver cells and P-388 tumor cells. The poor inhibition of protein synthesis in some tumors and normal liver cells, may be due to inability of the quassinoids to enter the cell, particularly in view of the fact that no inhibition was observed in whole cells of CF_1 liver, yet in the ribosomal fractionated system an ID_{50} of ~50 μM was observed. The observation that the quassinoids do not inhibit protein synthesis of all tumors may explain why bruceantin has not been observed in clinical trials to be effective against certain solid tumors. Perhaps it may be necessary to ascertain if the quassinoids are potent *in vitro* protein synthesis inhibitors of human solid tumors obtained by biopsy prior to administering the drug *in vivo*.

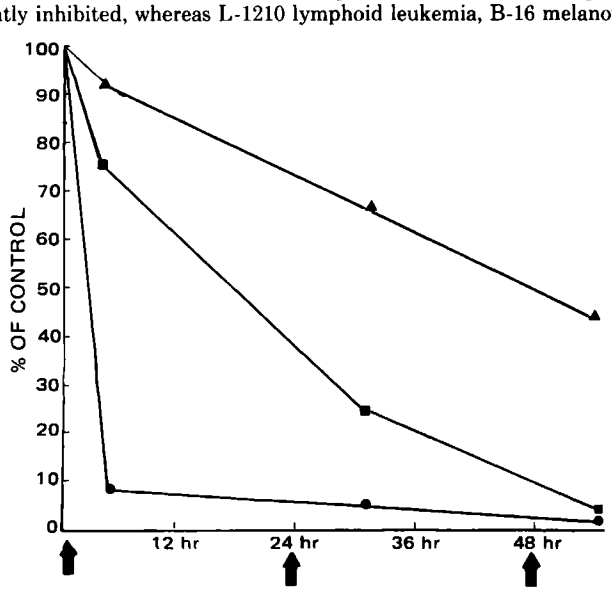

Figure 3—*The effect of (●) brusatol, (■) bruceantin, and (▲) bisbrusatolyl malonate on* in vivo *protein synthesis of P-388-NCI 6 hr after 1, 2, and 3 doses at 0.3 mg/kg ip.*

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High-Performance Liquid Chromatographic Assay of Methadone, Phencyclidine, and Metabolites by Postcolumn Ion-Pair Extraction and On-Line Fluorescent Detection of the Counterion with Applications

HARTMUT DERENDORF and EDWARD R. GARRETT ˣ

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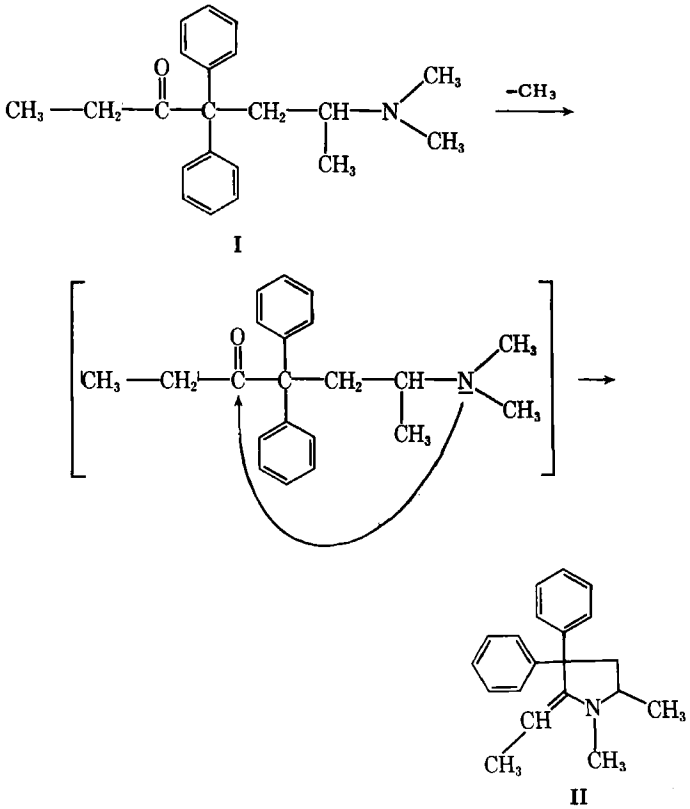
Abstract □ Methadone, phencyclidine, and their metabolites were extracted from plasma and separated on a high-performance liquid chromatographic (HPLC) column using the fluorescent 9,10-dimethoxyanthracene-2-sulfonic acid as a counterion. The chromatographed mobile phase was subsequently extracted on-line with chloroform. The separated organic phase, containing the fluorescent ion-pairs of the investigated amines, was analyzed in the flow cell of a fluorometer (excitation 380 nm, emission 445 nm). The phase separator volume was as small as possible to avoid dead volume. The method was also applied to the bioassay of cocaine with a sensitivity of 1–6 ng/ml of plasma. Application of these assays gave a red blood cell–plasma water partition coefficient for methadone of 3.39 ± 0.26 (*SD*) in a concentration range up to 20 μg/ml, and demonstrated a time-dependent partition with a diffusion half-life of 1.44 min ± 0.26 min (*SD*). The protein binding of methadone determined by ultracentrifugation was concentration dependent and varied between 75–62% at the highest concentration studied (9 μg/ml). The presence of the major metabolite did not have any influence on the protein binding. The results were confirmed by using the red blood cell-partitioning method to determine the protein binding.

Keyphrases □ Phencyclidine—high-performance liquid chromatographic assay of methadone and metabolites by postcolumn ion-pair extraction, on-line fluorescent detection of counterion with applications □ Methadone—high-performance liquid chromatographic assay of phencyclidine and metabolites by postcolumn ion-pair extraction, on-line fluorescent detection of counterion with applications □ High-performance liquid chromatography—assay of methadone, phencyclidine ion-pair extraction of counterion with applications

Classical high-performance liquid chromatography (HPLC) detection of drugs and their metabolites in biological fluids is by spectrophotometry and fluorescence. These methods were expanded by application of electrochemical detectors (1) and derivatization reactions (2) to get high sensitivities. Unfortunately, these direct detection methods are inadequate for some pharmacokinetic studies of drugs that are given in low doses and show low levels in biological fluids. Sensitive HPLC methods have not yet been developed (3) for methadone (I) and its major metabolite, 2-ethylidene-3,3-diphenyl-1,5-dimethylpyrrolidine (II) (Scheme I), nor for phencyclidine (III) and its hydroxylated metabolites (V, VI). These drugs have insufficient UV-absorbance, no fluorescence, and cannot be readily derivatized. Classical carbonyl reactions are not possible with methadone due to the steric effects of the two phenyl rings. Although cocaine (IV) can be spectrophotometrically detected in HPLC, greater sensitivity than the 15 ng/ml reported (4) would be advantageous in forensic and pharmacokinetic studies.



Scheme I—Major pathway of methadone metabolism.

All of these substances are tertiary amines which are readily protonated. They can be extracted into organic

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