

Microbial Transformations of Natural Antitumour Agents. Part 15.† Metabolism of Bruceantin by *Streptomyces griseus*

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Microbial transformations were conducted with the quassinoid bruceantin (1). Screening studies with 193 micro-organisms provided several streptomycetes capable of accumulating bruceantin metabolites. *Streptomyces griseus* (ATCC 10137) totally metabolized bruceantin within 19 h, and a preparative-scale incubation using bruceantin provided bruceine C (4), bruceantin 4',5'-epoxide (3), and bruceantin-5'-ol (5). Structures of metabolites were assigned based on their ¹H n.m.r. and mass spectral characteristics. Metabolites (3) and (5) are novel analogues of bruceantin, and are formed by an unusual pathway involving oxidation of bruceantin, probably through the unisolated 4',5'-bruceantin olefin (2) to the epoxide (3), which serves as an obligatory precursor for bruceine C (4) and bruceantin-5'-ol (5).

THE quassinoids¹ are bitter principles derived from the plant family Simaroubaceae. Many quassinoids exhibit promising antitumour activity,²⁻⁴ and bruceantin (1) obtained from *Brucea antidysenterica*³⁻⁵ is extremely active against L-1210 Lymphoid leukemia, the Lewis Lung carcinoma, and the B-16 melanocarcinoma,⁶ and the compound has been selected for clinical trial.^{7,8} The mode of action involves an irreversible inhibition of protein synthesis,⁹ and structure-activity relationships have been partially established for bruceantin and some of its analogues.¹⁰ While it appears that the bruceolide skeleton is required for antitumour activity, the nature of the C-15 ester grouping also plays an important role, possibly in the transport of bruceantin to critical sites within the cell. The structural complexity of bruceantin and related compounds has precluded the simple synthesis of additional analogues for study.

Microbial transformations have been extensively developed in our laboratories as tools for the preparation of potentially active metabolites of compounds like bruceantin, and for elaborating novel metabolic pathways by which such naturally occurring compounds undergo structural change in living systems.¹¹ This report describes the efficient production, isolation, and identification of three novel and related metabolites of bruceantin (1), by *Streptomyces griseus* (ATCC 10137).

EXPERIMENTAL

General.—Melting points were determined in open-ended capillary tubes in a Thomas-Hoover capillary melting-point apparatus and were corrected. U.v. spectra were recorded on a Beckman ratio-recording spectrophotometer; i.r. spectra were determined on a Beckman model 4240 spectrophotometer with KBr discs; and n.m.r. spectra were obtained in CDCl₃ with a Bruker 90 MHz or Varian T-60 spectrometer using SiMe₄ as internal standard. Low-resolution mass spectra were obtained with a Finnigan Model 3200 spectrometer and high-resolution mass spectra were obtained with a CEC 21-110B instrument through the mass-spectral services of the Department of Chemistry, Massachusetts Institute of Technology.

Bruceantin (1).—Bruceantin (NSC No. 165 563) was obtained from the National Cancer Institute, and had the

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following physical properties; m.p. 225–226 °C; [α]_D²⁵ –36° (c 2.0, pyridine); λ_{max} (EtOH) 222 (log ϵ 4.26), and 283 nm (3.90); λ_{max} (0.1N ethanolic NaOH) 221 (4.45) and 336 nm (3.66); ν_{max} (KBr disc) 3 442, 1 745, 1 665, 1 640, 1 438, 1 395, 1 360, 1 267, 1 215, 1 160, 1 058, 960, 825, and 742 cm⁻¹; n.m.r., see Table 1; *m/e* 548 (3%), 438 (7), 420 (1), 402 (3), 391 (2), 297 (2), 201 (2), 187 (2), 151 (4), 111 (100), 95 (4), 83 (3), 67 (3), and 55 (4); t.l.c., EtOAc, *R*_F 0.54. All these data correspond well with previously reported properties for bruceantin.^{5,8}

*Qualitative Determination of Epoxides.*¹²—A qualitative test was used to detect epoxide functional groups by treating samples (5–10 mg) in pure acetone (2 ml) with 0.1N sodium thiosulphate (2 ml) and one drop of phenolphthalein indicator. The solutions were warmed on the steam-bath and the development of a strong permanent red colour remaining for at least 1 h was considered a positive test. Metabolite (3) gave a positive test, while bruceantin, bruceine C (4), and bruceantin-5'-ol (5) were negative.

Chromatography.—Thin-layer chromatography was performed on 0.25- or 0.75-mm layers of silica gel GF₂₅₄ (Merck) prepared on glass plates with a Quickfit Industries spreader. Plates were air-dried and then oven activated at 120 °C for 30 min prior to use. Ethyl acetate (AnalaR) was used in developing t.l.c. plates, and compounds were visualized by fluorescence quenching under 254- or 365-nm u.v. irradiation, and were later visualized by spraying with 5% ethanolic FeCl₃. Bruceantin analogues reacted to give grey-black spots. The *R*_F values of bruceantin (1), bruceantin 4',5'-epoxide (3), bruceine C (4), and bruceantin-5'-ol (5) were 0.54, 0.48, 0.40, and 0.22 in this chromatographic system. Column chromatography was performed on silica gel (Baker 3 405) which was activated in an oven at 120 °C for 60 min prior to use. Columns were slurry packed in the developing solvent, and fractions were collected in a Fractometre 200 instrument, or manually.

High-performance liquid chromatography (h.p.l.c.) was performed with a Waters Associates ALC/GPC 202 instrument equipped with an M6000 solvent delivery system, a U6K universal injector and a 254-nm differential u.v. detector. Bruceantin and its metabolites were separated using a reversed-phase μ -Bondapak-Phenyl analytical column (Waters, 3.9 mm \times 30 cm), and a solvent system of MeCN–0.1% [(NH₄)₂CO₃ (1:1)] at a flow rate of 1.0 ml min⁻¹ and an operating pressure of 2 000 lb in⁻². Under these conditions, the retention volumes of bruceantin (1), bruceantin 4',5'-epoxide (3), bruceine C (4), and bruceantin-5'-ol (5) were 5.8, 4.5, 3.8, and 3.6 ml. The identities of each compound were confirmed by spiking crude extracts

with known compounds. H.p.l.c. analyses of cultures containing various bruceantin analogues were performed as follows. Incubations were adjusted to pH 5.5 for 24 h, extracted with equal volumes of EtOAc-BuⁿOH (9 : 1) and after clarification of the extracts by centrifugation, 10- μ l volumes were injected for analysis.

Fermentation Procedures.—Fermentations were conducted using the two-stage procedure and a soybean meal-glucose medium as previously described.¹³ Incubations were conducted on rotary shakers (Model G-25, New Brunswick Scientific Co.) operating at 250 r.p.m. and 27 °C. Bruceantin was added to 24-h old Stage II cultures as a solution in dimethylformamide to a final concentration of 500 μ g ml⁻¹ of culture medium. Screening experiments were conducted in 125-ml steel-capped Delong culture flasks holding 25 ml of medium. Control incubations were conducted to insure that bruceantin metabolites were not artifacts formed under the conditions prevailing during incubation. These controls consisted of cultures grown without bruceantin, and of flasks containing bruceantin in buffers at pH 2 and pH 3.4 (both 0.1M citrate-phosphate), pH 6.0 (0.066M phosphate) and pH 8.0 (0.2M Tris) without micro-organisms. Samples of incubations (4 ml) were withdrawn at various time intervals, adjusted to pH 5.5,

(3) was eluted with CHCl₃-MeOH (98 : 2) (120 ml) and was further purified by preparative t.l.c. and crystallization (EtOAc-hexane) to give fine white needles. Further elution with the same solvent (60 ml) and with CHCl₃-MeOH (9 : 1) (300 ml) gave metabolites (4) and (5), respectively. The latter two compounds were further purified by preparative layer t.l.c. using EtOAc as solvent, and (4) crystallized (MeOH) to fine white needles while (5) was obtained as an amorphous powder which resisted crystallization. Continued elution of the column with polar solvents resulted in the isolation of two minor and still unidentified bruceantin metabolites. The recoveries of pure compound from this column were bruceantin (1) (50 mg), metabolite (3) (200 mg), metabolite (4) (420 mg), and metabolite (5) (83 mg).

Characterization of Bruceantin 4',5'-Epoxide (3).—This metabolite had m.p. 223–226 °C; $[\alpha]_D^{25}$ -34.0 (*c* 1.0, pyridine); λ_{\max} (EtOH) 222 (log ϵ 4.05) and 280 nm (3.81); λ_{\max} (0.1N ethanolic NaOH) 214 (4.55) and 340 nm (3.72); ν_{\max} (KBr disc) 3 420, 1 730, 1 655, 1 635, 1 430, 1 385, 1 350, 1 265, 1 155, 1 100, 1 050, 960, 820, 790, and 740 cm⁻¹; n.m.r., see Table 1; high-resolution mass spectrum; *m/e* 562.202 08 (4%) for C₂₈H₃₄O₁₂ (calc. 562.205 03), 544 (22), 438 (55), 420 (30), 392 (59), 297 (47), 263 (49), 201 (42),

TABLE 1

¹H N.m.r. spectra of bruceantin (1), bruceantin 4',5'-epoxide (3), bruceantin-4'-ol (bruceine C) (4), and bruceantin-5'-ol (5). Spectra were taken in CDCl₃ and values are given in δ (*J* in Hz). Spectra for (1), (3), and (4) were taken on a 90-MHz instrument, while (5) was taken on a 60-MHz instrument

	(1)	(3)	(4)	(5)
4-Me	1.85 d(1.7)	1.85 d(1.6)	1.85 d(1.8)	1.85 d(1)
10-Me	1.39 s	1.39 s	1.38 s	1.35 s
H-15	6.21 d(12.6)	6.29 d(12.7)	6.30 d(12.6)	6.21 d(13)
OMe	3.77 s	3.79 s	3.79 s	3.75 s
H-2'	5.64 br, s	5.92 br, s	6.09 br, s	5.67 br, s
3'-Me	2.15 d(1)	2.19 d(1.2)	2.19 d(1.2)	2.19 d(1)
4'-Me	1.07 d(6)	1.49 s	1.38 s	1.05 d(6)
C-5'	1.07 d(6)	2.72 q(5.4)	1.38 s	3.60 d(6)

and extracted with EtOAc-BuⁿOH (9 : 1) (1.0 ml), and 30 μ l of the extracts were spotted on t.l.c. plates. Five species of *Streptomyces* reproducibly formed a major metabolite of bruceantin. *S. griseus* (ATCC 10137) produced the highest yields of metabolites in the shortest times, and this culture was selected to produce quantities of the metabolites for structure elucidation and biological evaluation.

Production of Metabolites (3), (4), and (5) from Bruceantin by *S. griseus*.—*S. griseus* (ATCC 10137) was grown in ten 1-l steel-capped Delong culture flasks each holding 200 ml of medium. Bruceantin (1) (1.0 g) was dissolved in dimethylformamide (10 ml) and distributed evenly among the 24-h old Stage II culture flasks, and incubations were monitored by t.l.c. to determine the rate and extent of conversion. The reaction was observable (20% t.l.c. estimate) within 2 h of incubation, and the fermentation was harvested for work-up 22 h after bruceantin addition.

The pooled cultures were adjusted to pH 5.5 with 2N HCl and exhaustively extracted with EtOAc-BuⁿOH (9 : 1) (6 l). The organic phases were combined and dried over anhydrous sodium sulphate and evaporated to dryness under vacuum to yield a brownish oil (2.042 g). The oil was adsorbed onto a silica gel column (90 g, 3 \times 33 cm) which was eluted at a flow rate of 5 ml min⁻¹ while 50-ml fractions were collected. Bruceantin (50 mg) was recovered following elution with CHCl₃ (360 ml). The first metabolite

187 (34), 154 (53), 151 (71), 138 (35), 125.058 91 (100) [for C₇H₉O₂ (calc. 125.060 25)], 121 (28), 112 (72), 98 (61), and 79 (59). The molecular ion was also evident by field-desorption mass spectrometry at *m/e* 562.

Properties of Bruceantin-4'-ol (4) or Bruceine C.—This metabolite had m.p. 176 °C; $[\alpha]_D^{25}$ -30.2 (*c* 0.63, pyridine); λ_{\max} (EtOH) 225 (log ϵ 4.09) and 282 nm (3.87); λ_{\max} (0.1N ethanolic NaOH) 214 (4.49) and 340 nm (3.69); ν_{\max} (KBr disc) 3 440, 1 730, 1 660, 1 635, 1 430, 1 390, 1 355, 1 260, 1 155, 1 055, 950, 830, 740, and 690 cm⁻¹; n.m.r., see Table 1; electron impact high-resolution mass spectrum; *m/e* 438 (20%), 418 (35), 392 (33), 297 (47), 201 (49), 151 (58), 127 (78), 111 (97), 101 (92), and 83 (100); the molecular ion was observable in the field-desorption mass spectrum at *m/e* 564 for C₂₈H₃₆O₁₂. The metabolite structure was comparable in all respects to bruceine C (4) provided as an authentic standard by Dr. J. Polonsky.

Properties of Bruceantin-5'-ol (5).—This metabolite remained amorphous; it had m.p. 186–190 °C; $[\alpha]_D^{25}$ -26.0 (*c* 1.0 pyridine); λ_{\max} (EtOH) 224 (log ϵ 4.07) and 282 nm (3.77); λ_{\max} (0.1N ethanolic NaOH) 214 (4.52) and 340 nm (3.68); ν_{\max} (KBr disc) 3 420, 1 735, 1 655, 1 630, 1 380, 1 355, 1 260, 1 200, 1 140, 1 050, 1 030, 825, and 725 cm⁻¹; n.m.r., see Table 1; high-resolution mass spectrum, electron impact, *m/e* 438 (29%), 392 (46), 297 (21), 201 (17), 151 (50), 127.074 81 [for C₇H₁₁O₂ (calc. 127.075 90)] (72), 96 (71), 83 (55), and 43 (100); field-desorption mass

spectrometry provided a molecular ion at m/e 564 for $C_{28}H_{36}O_{12}$.

RESULTS AND DISCUSSION

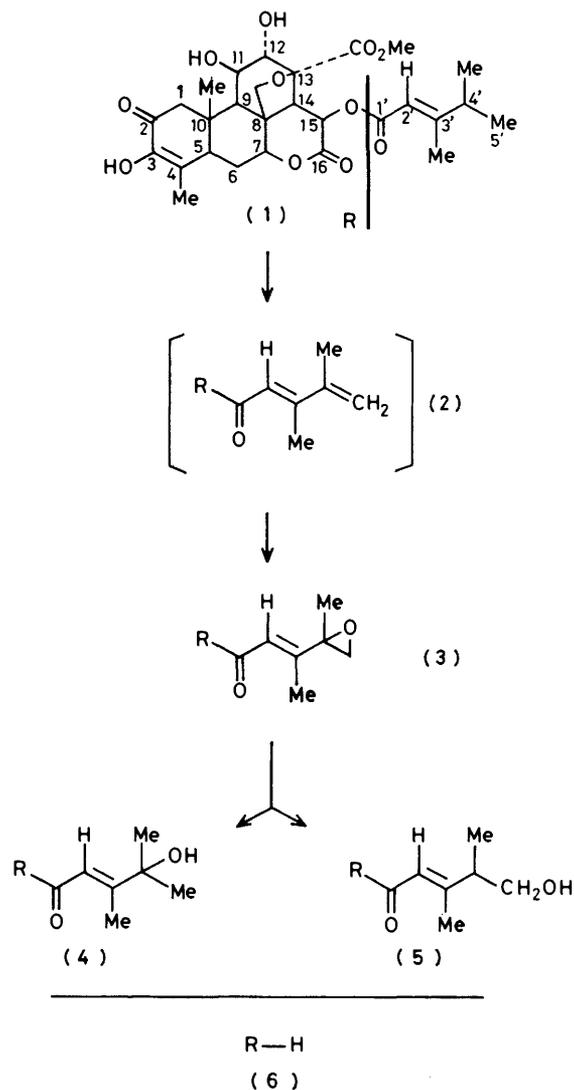
Because of its structural complexity, bruceantin (1) was an attractive candidate for microbial transformation study for the purposes of preparing new and active analogues, and for learning pathways by which the quassinoids undergo bio-transformations in living systems. The variety of functional groups present on the bruceolide (6) and 15-side-chain ester moieties would render simple chemical modifications difficult to achieve. *A priori*, several potentially useful enzymatic transformations were anticipated for bruceantin, including regioselective alcohol oxidation, selective ester hydrolyses, hydroxylations at allylic and other positions, opening of the furan ring following hydroxylation, and possibly combinations of these reactions.¹¹ A total of 193 cultures broadly representing yeasts, fungi, actinomycetes, and other bacteria were screened for their abilities to accumulate metabolites of bruceantin. Five streptomycetes reproducibly formed metabolites including *Streptomyces spectabilicus* (UI 632), *S. griseus* (UI 1158), *S. griseus* (ATCC 10137), *S. griseus* var. *erizensis* (NRRL 3242), and *S. aureofaciens* (ATCC 13304). These were the only cultures found to metabolize bruceantin, and the highest yields of products were formed by *S. griseus* ATCC 10137. This organism would form metabolites when used either as resting cells suspended in phosphate buffer, or in the growing state.

When *S. griseus* (ATCC 10137) was incubated with bruceantin (1.0 g), three major and two minor metabolites were formed. Metabolite production began in 2 h, and reached maximum levels 22 h after bruceantin addition. The metabolites were isolated by solvent extraction, and purified by chromatography and recrystallization to provide excellent yields of compounds (3) (200 mg), (4) (420 mg), and (5) (83 mg) while unreacted bruceantin starting material (50 mg) was also recovered. Traces of two very polar metabolites were obtained, but these were available in insufficient quantities for structure elucidation.

U.v. and i.r. spectral data for all the metabolites were comparable to bruceantin itself. All compounds displayed a large bathochromic shift of the 280 nm absorption which is attributable to the diosphenol group in ring A of bruceantin. Likewise, all compounds exhibited strong OH-stretching at 3400 cm^{-1} , a prominent band at 1740 cm^{-1} for the ester and lactone groups, 1660 cm^{-1} for the $\alpha\beta$ -unsaturated ketone of ring A, and a band at 1635 cm^{-1} for the double bonds.

Bruceantin¹⁴ and its metabolites displayed common intense mass-spectral fragments at m/e 151 and 438, attributed to cleavage of ring B, and elimination of the ester grouping at position 15. These simple fragmentations supported the u.v. and i.r. spectral results which suggested that the bruceolide (6) moiety remained unchanged in the structures of the metabolites. The

molecular ion of metabolite (3) at m/e 562.202 08 suggested an empirical formula of $C_{28}H_{34}O_{12}$ which would indicate the presence of an additional oxygen atom compared to bruceantin minus two hydrogens. Molecular ions for each of the other metabolites (4) and (5) required field-desorption mass-spectral treatment and revealed molecular weights of 564, or the addition of an



SCHEME Structures of bruceantin (1), microbial transformation products (3), (4), and (5), and bruceolide (6)

atom of oxygen for each compound. The base peak in bruceantin mass-spectral patterns occurs at m/e 111 for $C_7H_{11}O$, which corresponds to the ester side-chain from position 15. Metabolite (3) possessed a base peak at m/e 125 for $C_7H_9O_2$ while high-resolution mass spectra of metabolites (4) and (5) both displayed prominent fragments at m/e 127, corresponding to $C_7H_{11}O_2$. All these results provide evidence that the metabolic transformations achieved by *S. griseus* occurred on the 15-ester moiety.

Assignments of the positions and types of metabolically

introduced oxygen atoms were based on the n.m.r. spectral characteristics of the metabolites compared with bruceantin, which are summarized in Table 1. All four compounds displayed resonances corresponding to the bruceolide (6) skeleton which remained essentially unchanged. In addition, the vinyl methyl group (C-3') in the side-chain of bruceantin also appeared in each of the three metabolites at δ 2.19, coupled to the vinyl proton at C-2' which was also evident in the spectra of all metabolites and bruceantin. Major differences were found in the peaks assigned to the terminal isopropyl grouping of the ester side-chain. None of the meta-

structure was compared, by spectral and physical measurements, with an authentic sample of bruceine C which was isolated from *Brucea amarissima*,¹⁶ and which has recently been shown by X-ray analysis to contain a *trans*-2',3'-arrangement about the double bond of the side-chain ester.¹⁷

We initially considered the possibility that metabolite (4) might have been the compound named bruceantinol by Kupchan *et al.*⁵ However, bruceantinol clearly possessed the two distinct three-proton singlets at δ 1.40 and 2.02, assignable to the *gem*-dimethyl protons. Curiously the spectral data for bruceantinol were not

TABLE 2

Antitumour activities of the microbial metabolites of bruceantin in the P-388 lymphocytic leukaemia and B-16-melanoma carcinoma test systems

Compound	P-388				B-16			
	Dose per injection ^a (mg kg ⁻¹)	Survivors	Average days survival (T/C)	T/C ^b	Dose per injection (mg kg ⁻¹)	Survivors	Average days survival (T/C)	T/C ^b
(1)	2.0	6/10	6.3/11.4	Toxic	2.0	10/10	32.4/18.3	177
	1.0	10/10	23.3/11.4	204	1.0	10/10	29.0/18.3	158
	0.5	10/10	21.3/11.4	186	0.5	10/10	28.8/18.3	157
	0.25	10/10	21.8/11.4	191	0.25	10/10	28.3/18.3	154
	0.13	10/10	19.8/11.4	173	0.12	10/10	27.0/18.3	147
	0.06	10/10	17.3/11.4	151	0.06	10/10	25.3/18.3	138
(3)	0.50	2/6		Toxic	0.12	10/10	24.8/17.3	143
	0.25	6/6	13.8/10.4	132	0.06	10/10	22.8/17.3	131
	0.12	6/6	13.0/10.4	125	0.03	10/10	20.1/17.3	116
					0.01	10/10	18.7/17.3	108
(4)	16.0	0/6		Toxic	8.0	7/7	24.0/16.2	148
	8.0	5/6	18.0/11.1	162	4.0	10/10	22.3/16.2	137
	4.0	6/6	16.7/11.1	150	2.0	7/7	21.5/16.2	132
					1.0	10/10	19.3/16.2	119
(5)					0.5	10/10	17.9/16.2	110
	4.0	2/6		Toxic	4.0	9/10	34.0/18.4	184
	2.0	6/6	15.0/10.3	145	2.0	10/10	36.8/18.4	200
	1.0	6/6	16.0/10.3	155	1.0	10/10	31.0/18.4	168
	0.5	6/6	14.8/10.3	143	0.5	10/10	28.7/18.4	155

^a All compounds were prepared as suspensions in saline-ethanol and were administered intraperitoneally on a dosing schedule of once each day for a total of 9 doses beginning day 1 following tumour implantation. ^b T/C Values represent test (animals receiving drug) *vs.* control (animals receiving tumour implant only), and are based on the mean survival times of tumour-bearing mice. See ref. 18 for experimental protocols and further details on bioassay.

bolites displayed the *gem*-dimethyl group signals observable in bruceantin as a doublet at δ 1.07, J 6 Hz. The 4'-methyl group signal for metabolite (3) was a singlet at δ 1.49, while a 2-proton quartet at δ 2.72 was consistent with the presence of a 4',5'-epoxide.¹⁵ This was also the only metabolite which provided a positive qualitative epoxide test.¹² On the basis of mass-spectral and n.m.r. spectral evidence, the structure of (3) was assigned as bruceantin-4',5'-epoxide.

The second metabolite was assigned the structure of bruceantin-4'-ol (4) by mass and n.m.r. spectral analyses, and by comparison with authentic (4). Chemical shifts of the side-chain methyl group protons of (4) were nearly identical to those assigned for methyl *trans*-4-hydroxy-3,4-dimethylpent-2-enoate, which was synthesized by Kupchan and co-workers.⁵ The isopropyl six-proton doublet found in bruceantin was replaced by a six-proton singlet at δ 1.38 consistent with the presence of a tertiary alcohol substituent at position 4'. The metabolite

comparable to the synthetic methyl *trans*-4-hydroxy-3,4-dimethylpent-2-enoate ester side-chain. The vinyl C-2'-H signal of bruceantinol (δ 5.77) was also different to that of bruceine C (δ 6.09) and the pentenoate ester (δ 6.02). Comparisons of our metabolite with authentic standards of both bruceine C and bruceantinol obtained from the National Cancer Institute revealed that the two compounds were clearly different. Polonsky *et al.* have recently shown that the substance labelled bruceantinol is actually the same as bruceine-C-4'-acetate.¹⁷

The third metabolite produced by *S. griseus* was assigned the structure (5). The 4'-methyl signal occurs at δ 1.05 as a doublet (J 6 Hz), while the 5'-proton signals exist as a two-proton doublet at δ 3.60 (J 6 Hz). This is consistent with the presence of a primary alcohol functional group at the 5'-carbon atom of the metabolite.

A plausible metabolic pathway by which bruceantin could be enzymatically transformed into the identified

metabolites has been proposed (Scheme). We believe that the first step involves simple dehydrogenation of bruceantin to an unisolated olefin (2), followed by epoxidation to the 4',5'-epoxide (3). Enzymatic opening of the epoxide *via* hydride (*i.e.*, NADH, H⁺) attack would account for the ratio of metabolites (4) and (5) (5 : 1) obtained in all of our experiments. Attack at the primary site (position 5') would provide metabolite (4), while attack of a hydride ion at the more hindered 4'-position would give metabolite (5). Support for this hypothesis was obtained by using bruceantin, and metabolites (3), (4), and (5) each as substrates with *S. griseus* and by examining fermentation extracts by t.l.c. and h.p.l.c. Metabolite (3) was converted into (4) and (5), but the latter two metabolites remained unchanged after three days of incubation.

This is the first reported microbial transformation work with bruceantin. Enzymatic transformations performed by *S. griseus* are efficient and high yielding. Although (4) was the major metabolite and the previously known bruceine C, the two other derivatives are new analogues of bruceantin. All the metabolites retain the required ester portion of bruceantin,¹⁰ and biological evaluations of the metabolites were performed by the National Cancer Institute, Bethesda, Maryland, U.S.A., using the standard protocols¹⁸ for the B-16 melanocarcinoma and P-388 lymphocytic leukaemia test systems. The results of antitumour testing are presented in Table 2, where comparisons are made between each of the microbial metabolites (3), (4), and (5), and bruceantin (1).

None of the metabolites were as active as bruceantin *vs.* the P-388 test system. The least separation of toxicity *vs.* activity was found with the epoxide analogue (3) in the leukaemic system. Bruceine C (4) is much less active than bruceantin against P-388. In the B-16 system, only the primary alcohol derivative (5) displayed activity comparable with or better than bruceantin. Although the epoxide (3) was as active as bruceantin against the B-16 system at lower doses, repeated testing demonstrated that all test animals died at doses greater than 0.12 mg kg⁻¹. Bruceine C (4) is considerably less active than (1) in the B-16 system but it was not toxic at the doses examined.

It is interesting to suggest that bruceantin might undergo similar metabolic transformations when administered to mammals. Indeed the concept of micro-organisms serving as predictive models for mammalian metabolism has been well established.¹⁹

The work reported here serves to demonstrate that metabolites of bruceantin are not uniformly more toxic or less potent than bruceantin itself, and suggests further structure-activity relationships among the quassinoids related to bruceantin.

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