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INSECT ANTIFEEDANT AND GROWTH INHIBITORY ACTIVITY OF FORTY-SIX QUASSINOIDS ON TWO SPECIES OF AGRICULTURAL PESTS

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ABSTRACT.—Antifeedant and insect growth inhibitory activity of 46 natural and semi-synthetic quassinoids against tobacco budworm (*Heliothis virescens*) and black cutworm (*Agrotis ipsilon*) were compared to that of the known insect antifeedant azadirachtin. Structure/activity correlation indicates that cytotoxicity might be involved in the mode of action of these compounds.

The discovery of the potent antineoplastic activity of bruceantin [**21**], a quassinoid from *Brucea antidysenterica* (1), has generated much synthetic (2) and biological interest in this class of natural products from the Simaroubaceae (3,4). Apart from anticancer (5-15), antiviral (16), antiamoebic (17), antimalarial (18), and antiinflamatory (19) properties, quassinoids have been reported to be insecticidal (21) and to inhibit insect growth and feeding (20-22). In a study by the Native Plant Institute (20) activities of eight quassinoids on feeding and growth of fall armyworm (*Spodoptera frugiperda* J.E. Smith) and tobacco budworm (*Heliothis virescens* Fabr.) were examined. The structure/activity correlation followed closely the pattern recognized earlier in the cytotoxicity and antineoplastic activity studies. Thus, the A-ring Michael acceptor and the C-ring oxomethylene bridge were essential to elicit inhibition of growth. Presence of the ester sidechain seemed to be of lesser importance. The insecticidal activity on *Locusta migratoria* followed a similar structure/activity correlation pattern (21), while activity of 13 quassinoids on fall armyworm and Mexican bean beetle (*Epilachnia varivestis* Mulsant) studied under a different set of conditions (22) did not exhibit a consistent structure/activity relationship.

In the present communication, we report the effect of 46 quassinoids listed in Figure 1, on feeding of tobacco budworm (*H. virescens*) and development of black cutworm (*Agrotis ipsilon* Hafnagel). Their activity is compared to that of the well-known antifeedant and insect growth inhibitor azadirachtin [**1**] from *Azadirachta indica* Juss. (23,24).

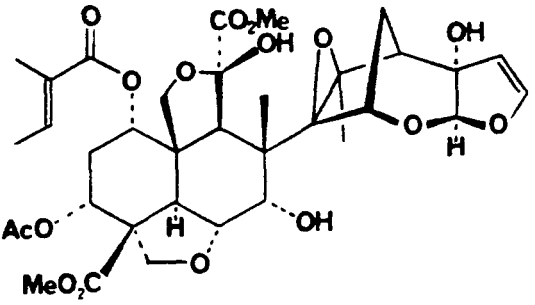
EXPERIMENTAL

TEST COMPOUNDS.—Quassinoids [**2**] (3,4), [**3-6**] (10,11), [**7-20**] (3,4), [**21**] (1,13), [**24-31**] (3,4), [**33-37**] (3,4), and [**44,45**] (13) were isolated in the pure state from natural sources.

Quassinoid analogue **32** was synthesized from **8** (25). Bruceolide [**23**] (3,4) was obtain by an alkaline hydrolysis of **3** (13); brucein-D triacetate [**47**] was prepared by acetylation of **4** with Ac_2O in pyridine (11); and compounds [**38-42**] were synthesized from **3**.[1]

BRUCEOSIDE-A ACETAL [**22**].—Treatment of bruceoside-A [**5**] (13) (100 mg) with acetaldehyde diethyl acetal (40 mg) in $CHCl_3$ (20 ml) containing *p*-toluene sulfonic acid (10 mg) at room temperature

[1]S. Tani, Y.M. Lin, and K.H. Lee, unpublished data.



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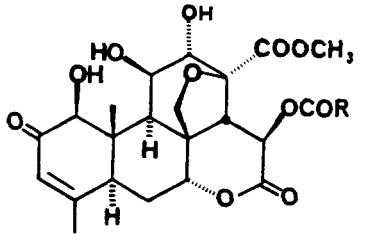
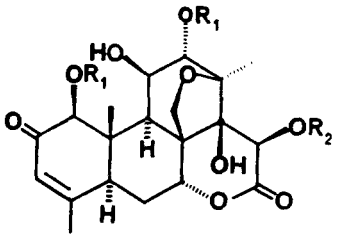
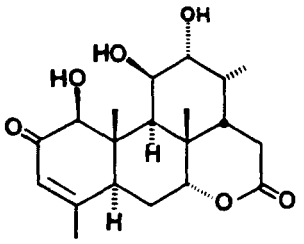


18 R=
28 R=CH_3
44 R=C_6H_5
45 R=



4 R_1=R_2=H
33 R_1=H, R_2=COC_6H_5
47 R_1=R_2=$COCH_3$
6 R_1=R_2=H, 2α-ol



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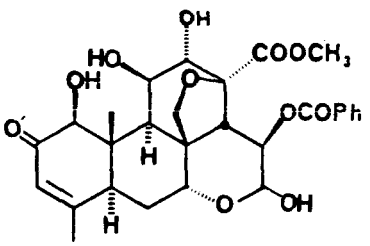

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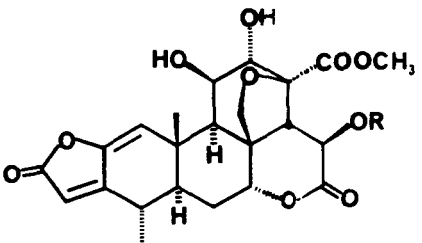
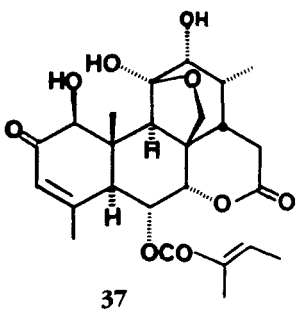


29 R=$COCH_3$
30 R=H



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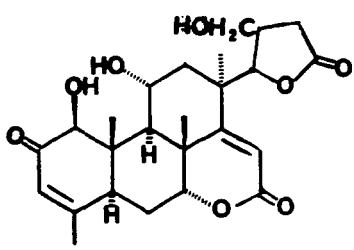
for 2 days, followed by Si gel preparative tlc of the resulting product, using $CHCl_3$-MeOH-Me_2CO (20:3:2) mixture of solvents, yielded bruceoside-A acetal [**22**] as amorphous powder (80 mg): mp 185-190°; ir cm^{-1} 3450 (br, OH), 1730-1760 (br, C=O) and 1640 (C=C); ^1H nmr (250 MHz, $CDCl_3$) ppm 1.17 (3H, d, J=7.0 Hz, *Me*CHOO), 1.60 (3H, s, Me-10), 1.39 (3H, d, J=5.0 Hz, Me-4) 1.6-2.1 (5H, m, H-5, H-6, H-9, H-14), 1.94 (3H, d, J=1.5 Hz, Me-23), 2.20 (3H, d, J=1.5 Hz, Me-23), 2.4-2.6 (1H, m, H-4) 3.35-3.80 (10H, m, sugar CH-O 6H+4OH), 3.81 (3H, s, COOMe) 4.15-4.28 (2H, m, H-11 and H-12), 4.44 (1H, br.s, H-15), 4.6-4.8 (4H, m, H-7, H-17 and anomeric H), 5.62 (1H, s, H-



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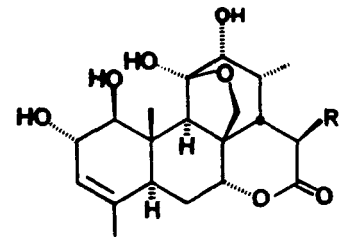
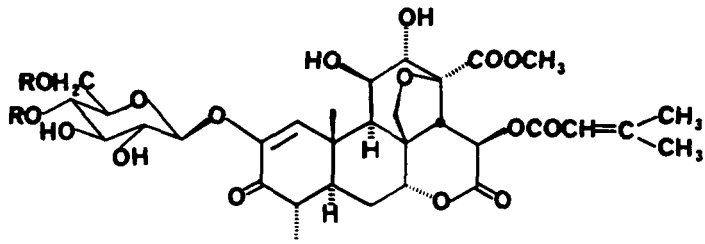
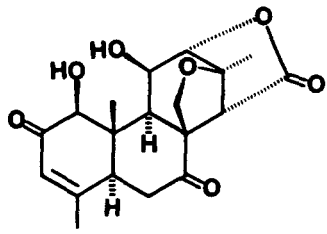


8 R=H
7 R=OH
9 R=OCO



5 R=H
22 R=



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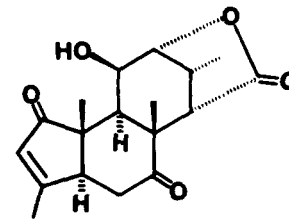

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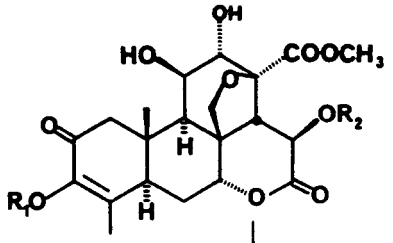
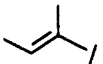
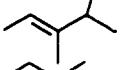
22), 6.20 (1H, br. Me*CH*OO) and 6.85 (1H, s, H-1). *Anal.* calcd. for $C_{34}H_{44}O_{16}$: *m/z* 708.2626. Found: *m/z* 708.2620.

16-HYDROXYBRUCEANOL-A [**46**].—To a solution of bruceanol-A [**44**] (13) (66 mg, 0.08 mmol) in MeOH (5 ml) was added a methanolic solution (0.6 ml) of $NaBH_4$ (3.1 mg, 0.08 mmol) at 0°. After the mixure was stirred at room temperature for 14 h, the MeOH was evaporated in vacuo. The residue was trituated with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was purified by preparative tlc and recrystallized from $CHCl_3$-Et_2O-hexane to afford **46** as an ammorphous powder (38 mg, 58%): mp 174-176°; ir cm^{-1} 3400 (OH), 1725 (ester CO) and 1670 (α,β-unsat. CO); ^1H nmr (250 MHz, $CDCl_3$) ppm 1.14 (3H, s, Me-10), 1.93 (3H, s, Me-4), 3.64 (3H, s, COOMe), and (1H, d, J=13 Hz, H-15). *Anal.* calcd for $C_{28}H_{32}O_{11}$: *m/z* 544.1945. Found: *m/z* 544.1949.

3-TRIFLUOROMETHANESULFONYLBRUCEANTIN [**43**].—To a solution of bruceantin [**21**] (1) (204 mg) in pyridine (1.5 ml), was added 1 ml of trifluoromethane sulfonic anhydride. After the solution was allowed to stand at room temperature for 1 h, it was poured into ice H_2O. The precipitate was filtered, dried, and purified by preparative tlc (Analtech, Si gel GF, 20×20 cm, 1000 μ, Rf 0.2) with $CHCl_3$-Et_2O-MeOH (10:8:1), to afford, after recrystallization from *n*-hexane-C_6H_6-$CHCl_3$, 58 mg of short needle crystals: mp 255-257°; ^1H nmr (250 MHz, $CDCl_3$) ppm 1.07 (6H, d, J=6.8 Hz, $CHMe_2$) 1.46 (3H, s, Me-10), 2.01 (3H, br.s, Me-4), 2.16 (3H, d, J<1.0 Hz, CH-*CMe*), 2.23 (1H, d, J=8.0 Hz, H-9), 3.78 (3H, s, COOMe), 3.82 (1h, d, J=8.0, H-17), 4.21 (1H, m, H-12), 4.28 (1H, m, H-11), 4.72 (1H, d, J=8.0 Hz, H-17), 4.83 (1H, m, H-7), 5.63 (1H, br.s, OCO-CH=C), and 6.21 (1H, br., H-15). *Anal.* calcd for $C_{29}H_{35}F_3O_{13}$: *m/z* 680.1750. Found: *m/z* 680.1688.

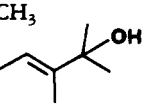


3 R_1=H, R_2=CO 

21 R_1=H, R_2=CO 

25 R_1=H, R_2=CO 

26 R_1=H, R_2=$COCH_3$

27 R_1=H, R_2=CO 

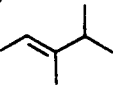
38 R_1=H, R_2=$COCHCH_2C_6H_5$ ($NH_2 \cdot HCl$)

39 R_1=H, R_2=$CONHCH_3$

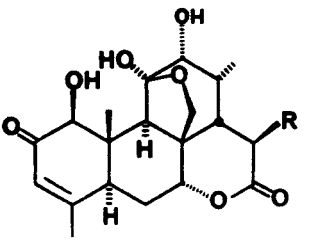
40 R_1=H, R_2=CO 

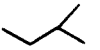
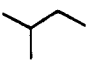
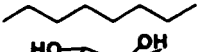
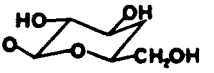
41 R_1=H, R_2=CO 

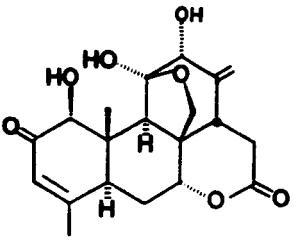
42 R_1=H, R_2=CO 

43 R_1=CF_3SO_2, R_2=CO 

23 R_1=R_2=H



10 R=H
11 R=OH
12 R=OCO 
13 R=OCO 
14 R=OCO 
32 R= 
34 R= 



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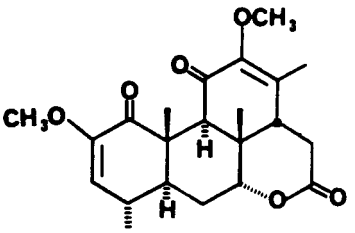

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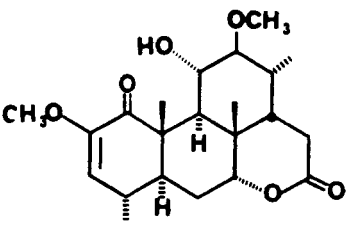

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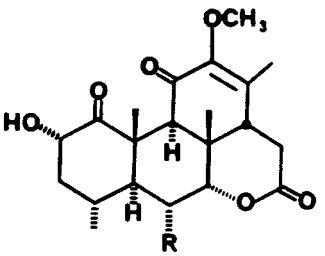
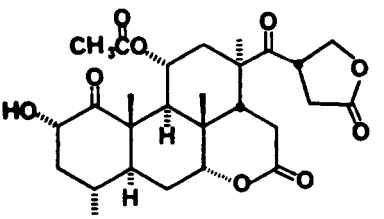


16 R=H
17 R=OH



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ANTIFEEDANT BIOASSAY.—Circular discs of 3-cm diameter punched out of cotton leaves were treated with a solution containing a known amount of active ingredient and then infested with 3rd instar tobacco budworm larvae (1 insect/disc). The percent feeding was determined visually 2 and 6 days after treatment. The check discs received blank solution containing all ingredients with the exception of the test compound. Feeding control was calculated according to the following formula: 100 (1-% feeding/% feeding by stock) and expressed on a scale ranging from one to three pluses. Three pluses corresponded to 90-100% control (excellent control), two pluses corresponded to 60-90% control, one plus to 30-60% control, and minus corresponded to 0-30% control (no control). The details of this bioassay have been described elsewhere (26).

TABLE 1. The Tobacco Budworm Antifeedant Activity of Quassinoids

	Compound	19.8 μg/cm²		12.0 μg/cm$_2$		6.0 μg/cm²		3.0 μg/cm²	
		2 days	6 days	2 days	6 days	2 days	6 days	2 days	6 days
1	azadirachtin	+++	++	+++	++	++	++	++	++
2	quassin	+	−						
4	brucein-D	+++	++	+++	++	+++	++	++	+
7	glaucarubol	+	−						
8	chaparrin	+	−						
9	glaucarubin	+	−						
11	glaucarubolone	++	+						
12	glaucarubinone	++	+						
14	ailanthinone	++	+						
16	picrasin-B	+	−						
17	6-hydroxypicrasin-B	+	−						
19	simarolide	+	−						
20	soulameolide	+	−						
21	bruceantin	+++	++	+++	++	+++	+	+++	+
24	simalikalacton-A	++	+	++	−	++	−	++	−
25	brucein-A	++	++	++	++	++	+	++	−
26	brucein-B	++	++	++	+	++	−	+	−
27	brucein-C	+++	++	++	+	++	−	+	−
28	isobrucein-B	++	++	++	−	++	−	++	−
29	sergolide	+++	+++	+++	+++	+++	++	+++	+
30	deacetyl sergolide	++	+	++	++	++	−	++	−
31	klaineanone	++	−	++	−	++	−	−	−
32	15-heptylchaparrinone	+++	++	++	++	++	++	+	−
33	15-*O*-benzoylbrucein-D	+++	++	+++	++	++	+	++	+
34	glucopyranosyl-glaucarubolone	++	−	+	−	+	−	−	−
35	samaderin	++	+	++	−	++	−	+	−
36	laurycolactone-A	−	−	−	−	−	−	−	−
37	6-tigloyl-chaparrinone	++	++	++	++	+	+	+	−
38	15-phenylalaninyl-bruceolide HCl			+	−	−	−	−	−
39	15-*N*-methyl carbamoyl-bruceolide			−	−	−	−	−	−
40	15-*p*-chlorobenzoyl-bruceolide			+	−	++	−	−	−
41	15-*m*-chlorobenzoyl-bruceolide			++	−	+	−	−	−
42	15-*O*-chlorobenzoyl-bruceolide			++	−	+	−	−	−
43	3-trifluoromethanesulfonyl-bruceantin			+	−	−	−	−	−
44	bruceanol-A			++	++	++	++	++	+
45	bruceanol-B			++	+	++	−	++	−
46	16-hydroxybruceanol-A			−	−	−	−	−	−
47	brucein-D triacetate			−	−	−	−	−	−

GROWTH INHIBITION BIOASSAY.—The standard screening rate of 30 ppm was achieved by admixing 3 mg of active ingredient dissolved in 0.5 ml of Me_2CO-DMSO (1:1) solution into a slurry of Bioserv Black Cutworm diet (100 ml) at 65°. This diet was then subdivided and exposed to 10 newly molted 4th instar black cutworms. The larvae were held at 26° 14:10 light dark cycle in 20 ml vials. The developmental abnormalities were monitored 2 days after the solvent treated control animals had pupated (14 days). Scores were reported as means. The scoring system was as follows: 0.0 normal pupae; 0.5 malformed pupa; 1.0 larval pupal intermediate; 2.0 delayed development, larvae in late last instar; 2.5 delayed development, larvae in early last instar; and 3.0 delayed development ; larvae in 4th instar (molt did not occur). Active compounds were titered down in dose.

RESULTS AND DISCUSSION

The results summarized in Tables 1 and 2 show that several quassinoids potently inhibit feeding and delay pupation of lepidopteran insects, even at low application levels. However, unlike azadirachtin, which is uniquely capable of ecdysis inhibition even below levels at which there is feeding inhibition (1 ppm of artificial diet), quassinoids do not elicit such effects even at high dose of 30 ppm. This suggests that the developmental delay might be due to the combination of starvation and toxicity. The discussion of structure/activity relationship below supports possible involvement of cytotoxicity in the mode of action of quassinoids.

The structure /activity correlation patterns for the feeding inhibition on tobacco

TABLE 2. Black Cutworm Growth Inhibitory Activity of Quassinoids

	Compound	Developmental score		Mortality/10 insects	
		30 ppm	10 ppm	30 ppm	10 ppm
1	azadirachtin	3.0	3.0		5
3	brusatol	0.7	0.6	0	0
4	brucein-D	2.0	1.8	1	0
5	bruceoside-A	0.6	0.2	0	0
6	brucein-E	2.0	0.2	1	0
7	glaucarubol	0.4		0	
8	chaparrin	0.0		0	
9	glaucarubin	0.1		0	
10	chaparrinone	2.0	1.33	0	0
11	glaucarubolone	1.1		0	
12	glaucarubinone	2.0	1.8	1	0
13	castelanone	2.0		0	
14	ailanthinone	2.45	2.0	0	0
15	ailanthone	2.0	1.7	0	0
16	picrasin-B	0.0		0	
17	6-hydroxypicrasin-B	0.0		0	
18	isobrucein-A	2.1	2.0	3	0
19	simarolide	0.0		0	
20	soulameolide	0.0		0	
21	bruceantin	2.0	2.0	0	0
22	bruceoside-A acetal	0.8	0.0	0	0
23	bruceolide	1.4	1.2	0	0
24	simalikalactone-A	0.0		0	
25	brucein-A	1.9		0	1
26	brucein-B	0.0		0	
27	brucein-C	0.1		0	
28	isobrucein-B	1.4		0	
29	sergolide	1.2		0	
30	deacetylsergolide	0.85		0	
31	klaineanone	1.6		0	

budworm and growth inhibition on black cutworm are very similar and can be summarized as follows: (a) The A-ring enerone function is essential to activity. Reduction of the electrophilic capacity of this Michael acceptor results in lowering of activity. Thus, the A-ring diosphenols are on the whole less active (cf. **25** versus **18**, Table 2). However, higher electrophilicity of diosphenol achieved by placement of the electron withdrawing trifluoromethyl sulfonyl substituent onto the 3-hydroxy group did not result in increased activity (cf. **43** in Table 1). (b) The C-ring oxomethylene bridge is very important (compound **31** has poor activity); C8 to C13 linkage seems to be somewhat more advantageous than C8 to C11 (cf. **4** versus **11** and **14** in Table 1). (c) Ester sidechains have in many cases great influence on activity (cf. **25** versus **26**). On the whole, hydrophilic sidechains seem to be detrimental (cf. **21** versus **27** in Tables 1 and 2; **40**, **41**, and **42** versus **38** and **39** in Table 1) while hydrophobic, unsaturated sidechains improve activity (cf. **44** and **45** in Table 1). Compounds lacking a sidechain altogether can be fairly active (cf. **4** in Table 1).

These results confirm the previously reported structure/activity correlation pattern (20,21). Great similarities in the structure/activity relationships suggest that cytotoxicity might play an important role in the toxicity of quassinoids to insects.

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