Metabolites of the Prototype Insecticide (2*E*,4*E*)-*N*-Isobutyl-6-phenylhexa-2,4-dienamide. 1. Synthesis, Chromatography, Spectroscopy, and Biological Activity

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Ten candidate metabolites of the prototype insecticide (2*E*,4*E*)-*N*-isobutyl-6-phenylhexa-2,4-dienamide (1) are prepared via a hydrozirconation procedure for stereospecific formation of the (2*E*,4*E*)-diene unit. This involves coupling vinylzirconocenes (derived from appropriately protected terminal acetylenes and dicyclopentadienylzirconium chloride hydride) with vinyl halides under palladium(0) catalysis in 38–57% yield. Standard deprotection and functionalization methodology yield the β-hydroxyisobutyl, 6-hydroxy, 6-keto, and *p*-hydroxy derivatives of **1**, the corresponding carboxylic acid and amide and their 6-hydroxy derivatives, and the β-hydroxyisobutyl, 6-hydroxy derivative of **1**. The hydroxamic acid is prepared by condensing *N*,*O*-bis(trimethylsilyl)hydroxylamine with the acid chloride followed by hydrolysis. HPLC and GC–MS readily distinguish **1** and its derivatives (or their methylation products) for metabolite analysis. Each of the candidate metabolites synthesized is less than one-third as toxic as **1** to piperonyl butoxide pretreated houseflies by injection and to mice by intraperitoneal administration.

Natural and synthetic isobutylamides [*N*-(2-methylpropyl)amides] have been studied as candidate insecticides for almost 40 years (Crombie, 1952; Jacobson, 1971; Miyakado et al., 1979, 1983; Elliott, 1985; Su, 1985). (2*E*,4*E*)-*N*-Isobutyl-6-phenylhexa-2,4-dienamide (**1**) is one of the most potent compounds examined and serves as a suitable prototype for further investigations (Elliott, 1985; Elliott et al., 1986, 1987a). Continuing structural optimization of the isobutylamide insecticides might be facilitated by knowledge of their metabolically labile sites leading to structural modifications to prolong their biological stability.

The metabolic hydroxylation reactions of isobutylamide **1** are expected to involve the α- and β-positions of the isobutyl group and the 6- and para-positions of the acid moiety and combinations thereof (Figure 1). The amide, which is potentially formed on breakdown of the α-hydroxy compound, may then undergo enzymatic cleavage to the carboxylic acid, either by direct means or via the hydroxamic acid. The 6-keto compound is also a candidate metabolite formed on oxidation of the 6-hydroxy compound. This report describes the synthesis, chromatography, spectroscopic properties, and biological activity of these candidate metabolites of **1** designated as shown in Figure 1.

SYNTHESES

Analytical Methods and Abbreviations. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker WM-300 instrument operated at 300 MHz with $CDCl_3$ as the solvent, unless otherwise stated. Chemical shifts (δ) are reported relative to tetramethylsilane (s, singlet; d, doublet; dd, double doublet; t, triplet, m, multiplet; br, broad). Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The conditions and procedures for high-performance liquid chromatography (HPLC), gas chromatography (GC), GC–mass spectrometry (MS), and chemical ionization mass spectrometry (CI–MS) are defined later and by Johnston et al. (1989). Masses are given for the quasi-molecular ions $[M + 1]^+$; the $[M + 29]^+$ and $[M + 41]^+$ signals were also monitored for confirmation.

Abbreviations used for substituents are Ar for aromatic, Bz for benzyl, and Me for methyl.

Synthesis Procedures. Analytical thin-layer chromatography (TLC) was carried out on precoated Polygram silica G/UV_{254} plastic sheets (0.25-mm layer thickness) (Machery-Nagel, Düren, Germany). Column chromatography utilized silica gel 60 F_{254} (Merck, Darmstadt, Germany) or neutral alumina (ICN alumina TSC; ICN, Eschwege, Germany). Benzene and tetrahydrofuran (THF) were distilled from sodium–benzophenone ketyl immediately prior to use. Acetone and CH_2Cl_2 were dried over molecular sieves (4 Å). Ether and hexane were dried over sodium wire. *N*,*N*-Dimethylformamide (DMF) was dried and distilled from $CaSO_4$. All reactions involving dry solvents were performed under nitrogen. During workup, solutions were dried over $MgSO_4$ and concentrated in vacuo.

General Procedures. Three approaches (A–C in Figure 2) were taken to the coupling of the appropriate terminal acetylenes (**5–7**) with the vinyl halides (**8–10**). Acetylenes **5** and **6** were prepared by literature procedures (Mulvaney et al., 1967; Pincock and Somawardhana, 1978). Compound **7** was synthesized by stirring phenylethinylcarbinol (Jones et al., 1956) for 1 h with *tert*-butyldimethylsilyl (TBDMS) chloride (Aizpurua and Palomo, 1985) in dry benzene using 1,8-diazabicyclo[5.4.0]undec-7-ene as a base to give the desired TBDMSO ether as a colorless oil: 82%; bp 92–95 °C (0.8 mmHg); MS, $[M + 1]^+$ 246; NMR 0.14 (3 H, s), 0.18 (3 H, s), 0.94 (9 H, s), 2.52 (1 H, d, J = 2.2 Hz), 5.48 (1 H, d, J = 2.2 Hz), 7.25–7.36 (5 H, m). Iodo amide **8** was prepared from propiolic acid. Thus, hydrobromination of the acid gives exclusively 3-bromoprop-2(*E*)-enoic acid (Yates and Auksi, 1979), which was further converted by treatment with phenyl *N*-phenylphosphoramidochloridate and isobutylamine (Mestres and Palomo, 1982) to the corresponding bromo amide, *N*-isobutyl-3-bromoprop-2(*E*)-enoic amide: MS, $[M + 1]^+$ 206; NMR 0.93 (6 H, d, J = 6.7 Hz), 1.81 (1 H, sept, J = 6.7 Hz), 3.15 (1 H, t, J = 6.7 Hz), 5.54 (1 H, br s), 6.48 (1 H, d, J = 13.4 Hz), 7.47 (1 H, d, J = 13.4 Hz). Finally, the bromo amide was treated with sodium iodide in acetone (Blade, 1987) catalyzed by aluminum chloride to give the desired iodo compound **8**: MS, $[M + 1]^+$ 254; NMR 0.93 (6 H, d, J = 6.5 Hz), 1.70 (1 H, sept, J = 6.5 Hz), 3.15 (2 H, t, J = 6.5 Hz), 5.65 (1 H, br s), 6.90 (1 H, d, J = 14.8 Hz), 7.76 (1 H, d, J = 14.8 Hz). Benzylation of the bromo acid using benzyl bromide in dry dimethylformamide (DMF) in the presence of anhydrous K_2CO_3 (Osborne, 1980) gave, after workup and purification by dry-column chromatography, benzyl ester **9** as a colorless oil: MS, $[M + 1]^+$ 240; NMR 5.13 (2 H, s), 6.51 (1 H, d, J = 13.8 Hz), 7.32 (5 H, m), 7.58 (1 H, d, J = 13.8 Hz). Methyl ester **10** was prepared by a literature procedure (Miyakado et al., 1979).

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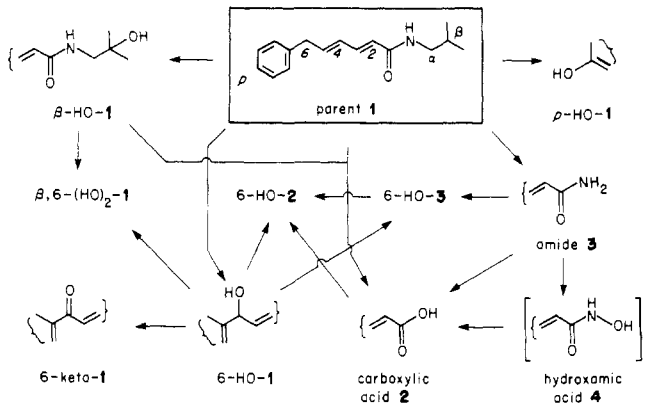

Figure 1. Metabolic reactions of isobutylamide 1 (Johnston et al., 1989) and designations used for the metabolites.

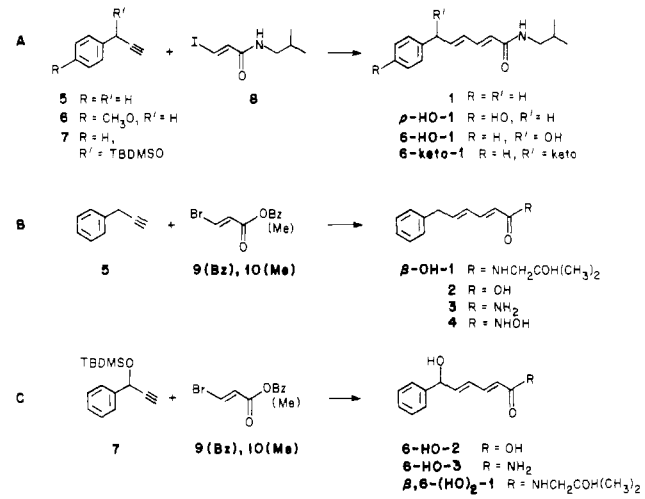

Figure 2. Synthesis of isobutylamide 1 and its candidate metabolites.

The coupling reaction involved hydrozirconation (Crombie et al., 1987), with dicyclopentadienylzirconium chloride hydride ($Cp_2ZrCl(H)$, Schwartz's reagent), of terminal acetylenes **5–7**, followed by palladium(0)-catalyzed coupling with vinyl halides **8–10** to give typical yields of 38–57% of coupled product. As a general procedure, the terminal acetylene (1 mmol) in dry benzene (10 mL) was treated with $Cp_2ZrCl(H)$ (1.2 mmol) and the resultant mixture stirred at room temperature for 4 h with the exclusion of light under nitrogen. Bis(triphenylphosphine)palladium dichloride (10 mol %) in dry THF was treated with a 1 M hexane solution of diisobutylaluminum hydride (DIBAL-H) (2 equiv) at 0 °C over 10 min to give a blackish-brown suspension, which was treated at 0 °C with the zirconocene solution, prepared above, and the vinyl halide (1 mmol). After being stirred overnight in the dark, the mixture was poured into 2 M aqueous HCl and extracted with ether. The combined extracts were washed with saturated $NaHCO_3$ solution and brine, dried, and concentrated. The resultant mixture was purified by dry-column chromatography on silica gel or neutral alumina to obtain the desired coupled product.

Scheme A. Coupling of **5** with **8**, by the hydrozirconation methodology, and purification (silica gel, ether/hexane (1:1)) gave isobutylamide **1** as a white solid (38%).

In a similar manner, coupling of **6** with **8** and purification (alumina, ether/hexane (2:3)) gave the natural product piperovatine: 40%; mp 123–124 °C (benzene/hexane); lit. mp 121 °C (Price and Pinder, (1970); MS, $[M + 1]^+$ 274; NMR 0.91 (6 H, d), 1.78 (1 H, sept), 3.15 (2 H, t), 3.41 (2 H, d), 3.79 (3 H, s), 5.64 (1 H, br s), 5.78 (1 H, d, J = 15.0 Hz), 6.12–6.19 (2 H, m), 6.95 (4 H, A_2B_2 pattern), 7.21 (1 H, dd, J = 15.0 Hz, 10.0 Hz). For demethylation (McOmie et al., 1968), a 1 M solution of boron tribromide (BBr_3) (1.5 mmol) in CH_2Cl_2 was added to a solution of piperovatine 0.37 mmol) in dry CH_2Cl_2 (2 mL). After 2 h, the reaction was quenched by careful addition of water and then the

Table I. Nuclear Magnetic Resonance Data for Isobutylamide 1 and Its Candidate Metabolites

$(CH_3)_2CHCH_2NHC(O)CH{=}CHCH{=}CHCH_2Ar$
$\gamma\ \ \beta\ \ \alpha\ \ \ \ 2\ \ 3\ \ 4\ \ \ 5\ \ 6$

compd[a]	proton assignments for 1 and metabolites thereof: δ (J, Hz)								
	H_α	H_β	H_γ	H_2	H_3	H_4	H_5	H_6	Ar
1	3.15 ($J_{\alpha,\beta}$ = 6.5)	1.79	0.91 ($J_{\beta,\gamma}$ = 6.5)	5.80 ($J_{2,3}$ = 14.9)	*b*	6.14[c] ($J_{4,5}$ = 14.9, $J_{3,4}$ = 9.2)	6.21[c]	3.48 ($J_{5,6}$ = 5.5)	7.14–7.33
p-HO-1	3.07 ($J_{\alpha,\beta}$ = 6.6)	1.76	0.88 ($J_{\beta,\gamma}$ = 6.6)	5.99 ($J_{2,3}$ = 15.0)	7.15	6.20[d] ($J_{4,5}$ = 16.2, $J_{3,4}$ = 11.0)	6.17[d]	3.39[d] ($J_{5,6}$ = 5.8)	6.77, 7.02 (J = 8.5)
6-HO-1	3.16 ($J_{\alpha,\beta}$ = 6.6)	1.80	0.92 ($J_{\beta,\gamma}$ = 6.6)	5.89 ($J_{2,3}$ = 15.0)	7.22	6.43 ($J_{4,5}$ = 15.3, $J_{3,4}$ = 11.0)	6.21	5.31 ($J_{5,6}$ = 5.6)	7.29–7.37
6-keto-1	3.21 ($J_{\alpha,\beta}$ = 6.5)	1.84	0.95 ($J_{\beta,\gamma}$ = 6.6)	6.34[d] ($J_{2,3}$ = 14.0)	7.47[d] ($J_{3,4}$ = 10.1)	7.45[d]	7.27[d] ($J_{4,5}$ = 14.0)		7.36–7.63 (3 H), 7.94–7.97 (2 H)
β-HO-1	3.33 ($J_{\alpha,N\text{-}H}$ = 6.1)	1.22		5.84 ($J_{2,3}$ = 15.0)	*b*	6.15[c] ($J_{4,5}$ = 15.0, $J_{3,4}$ = 9.2)	6.23[c]	3.48 ($J_{5,6}$ = 5.6)	7.14–7.33
β,6-(HO)$_2$-1	3.33 ($J_{\alpha,N\text{-}H}$ = 6.1)	1.22		5.94 ($J_{2,3}$ = 15.0)	7.23	6.44 ($J_{4,5}$ = 15.4, $J_{3,4}$ = 11.0)	6.22	5.31 ($J_{5,6}$ = 5.6)	7.25–7.36
2				5.81 ($J_{2,3}$ = 15.4)	*b*	6.22[c] ($J_{4,5}$ = 5.1, $J_{3,4}$ = 10.1)	6.32[c]	3.51 ($J_{5,6}$ = 6.2)	7.15–7.40
6-HO-2				5.90 ($J_{2,3}$ = 15.4)	*b*	6.47 ($J_{4,5}$ = 15.0, $J_{3,4}$ = 11.1)	6.26	5.24 ($J_{5,6}$ = 5.8)	7.18–7.38
3				5.91 ($J_{2,3}$ = 15.2)	*b*	6.19[c] ($J_{4,5}$ = 15.0, $J_{3,4}$ = 9.1)	6.27[c]	3.50 ($J_{5,6}$ = 5.6)	7.14–7.46
6-HO-3				6.02 ($J_{2,3}$ = 15.2)	7.19	6.44 ($J_{4,5}$ = 15.1, $J_{3,4}$ = 11.0)	6.25	5.26 ($J_{5,6}$ = 5.7)	7.26–7.36
4				5.79 ($J_{2,3}$ = 15.2)	*b*	6.14–6.30	6.14–6.30	3.48 ($J_{5,6}$ = 5.3)	7.12–7.30

[a] In $CDCl_3$ except for *p*-HO-1 in acetone-d_6, **3** and **6-HO-3** in $CDCl_3/CD_3OD$, and **6-HO-2** and **4** in CD_3OD. [b] Resonance obscured in aromatic region. [c] Measured from homonuclear decoupled (irradiation at H_6) spectrum. [d] Coupling constant determined from spin simulation program.

Table II. Chromatographic and Chemical Ionization Mass Spectral Properties of Isobutylamide 1 and Its Candidate Metabolites

compd	mp, °C	HPLC[a] R_t, min	GC R_t, min SD 54[b]	GC R_t, min SPB5[c]	CI-MS m/e (rel intens)[d] [M + 1]$^+$	CI-MS m/e (rel intens)[d] imp frag
1	119–121[e]	61.0	21.6	16.8	244 (100)	
p-HO-1	101–102	35.0	(28.6)[f]	(25.2)[f]	260 (100)	
6-HO-1	105–107	48.5	27.1	23.7	260 (100)	244 (40), 242 (40)
6-keto-1	128–130[g]	58.0	27.4	23.8	258 (100)	
β-HO-1	78–79	46.0	25.1	20.8	260 (100)	242 (60)
β,6-$(HO)_2$-1	oil	19.5		19.0	276 (50)	258 (100)
2	89–90	11.0	(9.6)[f]	(6.6)[f]	189 (100)	
6-HO-2	129–130	5.0		(9.2)[f]	205 (50)	187 (100)
3	165–167	41.5	14.9	10.2	188 (100)	
6-HO-3	146	15.0		12.7	204 (100)	188 (60)
4	131–134	*h*		*i*	204 (100)	188 (60)

[a] Altex Ultrasphere-Si column (4.6 mm × 25 cm; Altex Scientific Inc., Berkeley, CA) with a gradient of 38% methanol (HPLC grade) in water (HPLC grade) to 100% methanol over a period of 50 min followed by 100% methanol for an additional 30 min with a constant flow rate of 0.8 mL/min at 25 °C. [b] Fused silica capillary column (0.25 mm × 15 m; Supelco, Inc., Bellefonte, PA) with SE 54 (0.25-μm film thickness) and a temperature program of 90 °C for 3 min followed by 25 °C/min to 165 °C, 165 °C for 1 min, and then 3 °C/min to 240 °C with helium as the carrier gas at 40 cm/s using a flame ionization detector. [c] Fused silica capillary column (0.32 mm × 15 m; Supelco) with SPB5 (0.32-μm film thickness) and a temperature program of 90 °C for 3 min followed by 25 °C/min to 160 °C, 160 °C for 1 min, and then 2 °C/min to 240 °C with helium as the carrier gas at 30 cm/s using the MS total ion current as the detector. [d] The relative signal intensities vary to some extent on introducing the sample by GC or via direct insertion and on changing the methane pressure or source temperature. [e] Lit. mp 112–114 °C (Elliott et al., 1987a). [f] GC data: methyl ether of p-HO-1, [M + 1]$^+$ 274; methyl ester of 2, [M + 1]$^+$ 203; methyl ester of 6-HO-2, [M + 1]$^+$ 219. [g] Lit. mp 105–110 °C (Elliott et al., 1987b). [h] Poor peak shape probably due to chelation with iron impurities (Corbett and Chipko, 1979). [i] Decomposes in the GC injection port to give 3 and two other products ([M + 1]$^+$ 186) with R_t 5.7 and 5.9 min.

mixture was extracted with ether. The ether extracts were combined, washed with water (2×) and brine, dried, and concentrated. Purification (silica gel, ether/hexane (2:1)) gave p-HO-1 as a white solid (73%).

Coupling acetylene **7** with iodo amide **8** gave, after purification (silica gel, ether/hexane (1:1)), a mixture of the required product 6-TBDMSO-1 and iodo amide **8**, as shown by NMR. Without further purification, desilylation of the mixture with tetrabutylammonium fluoride in dry THF followed by purification (silica gel, ether/hexane (4:1)) furnished 6-HO-1 as a white solid (overall yield 38%).

Oxidation of 6-HO-1 with MnO_2 in dry CH_2Cl_2 (Barrelle and Glenat, 1967), followed by filtration and concentration, gave essentially the desired 6-keto-1 which was obtained pure (48%) after chromatography (silica gel, ether/hexane (4:1)).

Scheme B. Coupling of acetylene **5** with bromo ester **9**(Bz), via the hydrozirconation methodology, and purification (silica gel, ether/hexane (1:5)) gave **2**(Bz) as a white solid: 40%; mp 56 °C (pentane); MS, [M + 1]$^+$ 279; NMR 3.46 (2 H, d, J = 5.7 Hz), 5.16 (2 H, s), 5.85 (1 H, d, J = 15.4 Hz), 6.15–6.31 (2 H, m), 7.12–7.35 (11 H, m). Hydrolysis of **2**(Bz) to acid **2** was achieved with use of BBr_3 (Bhatt and Kulkarni, 1983). To a solution of the ester (0.2 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise 1 M BBr_3 (2 mmol) in CH_2Cl_2 solution at −10 °C. The reaction was kept at −10 °C for 1 h and at 25 °C for 2 h, before it was terminated by careful addition to water. The layers were separated, and the organic phase was washed with water (2×), dried, and concentrated to give essentially the desired acid **2** as indicated by TLC analysis. Further purification (silica gel, ethyl acetate/hexane (1:2)) gave pure **2** (84%).

An alternative procedure was also examined for preparation of **2** whereby acetylene **5** was coupled with bromo ester **10**(Me). Purification (silica gel, hexane) gave **2**(Me) as a colorless oil: 48%; MS, [M + 1]$^+$ 203; NMR 3.45 (2 H, d, J = 5.6 Hz), 3.69 (3 H, s), 5.80 (1 H, d, J = 15.4 Hz), 6.11–6.27 (2 H, m), 7.11–7.32 (6 H, m). However, **2**(Me) could not be hydrolyzed without isomerization; i.e. hydrolysis in dioxane, concentrated HCl, and water (Price and Pinder, 1970; Blade and Robinson, 1986) gave acid **2** contaminated with its isomers. Alkaline hydrolysis was also unsuitable since the 4,5-double bond shifts into conjugation with the aromatic nucleus (Winterfeldt, 1963). In the case of **2**(Bz), no isomerization is detected by ^1H and ^{13}C NMR on hydrolysis with BBr_3.

β-HO-1 was prepared by condensing 2-hydroxyisobutylamine (Aihara, 1951; DeJongh et al., 1975) with the acid chloride of **2**. Thus, acid **2** (0.27 mmol) in dry ether (5 mL) was treated with oxalyl chloride (1 mL), and the mixture was kept at room temperature overnight under nitrogen. The solvent and excess oxalyl chloride were removed in vacuo, and the residue was dissolved in dry ether (10 mL) and then cooled in ice. A solution of 2-hydroxyisobutylamine (2.7 mmol) in dry ether was added gradually. After about 1 h the reaction was complete as indicated by TLC analysis. The mixture was poured into water and extracted with ether. The combined extracts were washed with brine, dried, and concentrated. Purification (silica gel, ethyl acetate/hexane (4:1)) gave β-HO-1 as a white solid (51%).

2(Me) was directly converted to primary amide **3** with the aluminum amide reagent derived from trimethylaluminum and ammonium chloride (Levin et al., 1982). To a suspension of ammonium chloride (1.45 mmol) in dry benzene (1.5 mL) at 5 °C was slowly added a 2 M solution (1.54 mmol) of trimethylaluminum in toluene. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred for a further 3 h. To a solution of **2**(Me) (0.47 mmol) in dry benzene (5 mL) was added the ammonium reagent prepared above. The solution was heated under nitrogen at 55 °C for 16 h. The reaction mixture was cooled to room temperature and carefully quenched with 5% HCl. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3×). The organic extracts were combined, dried, and concentrated to afford essentially amide **3**. Further purification (silica gel, ethyl acetate/hexane (9:1)) gave the primary amide as a white solid (68%). The same procedure applied to **2**(Bz) also gave **3** but in lower yield (38%).

Hydroxamic acid **4** was synthesized by condensation of *N,O*-bis(trimethylsilyl)hydroxylamine with the acid chloride from **2** (King et al., 1978). Thus, acid **2** (0.48 mmol) in dry ether (2 mL) was treated with oxalyl chloride (2 mL) and the mixture kept at room temperature overnight under nitrogen. The solvent and excess oxalyl chloride were removed in vacuo. The residual yellow oil was again diluted with dry ether (2 mL), and *N,O*-bis(trimethylsilyl)hydroxylamine (1 mmol) was added dropwise at room temperature. After 1 h, the reaction mixture was concentrated and the residue diluted with methanol to effect methanolysis. TLC analysis (chloroform:methanol = 19:1, detection with $FeCl_3$) confirmed the clean formation of the hydroxamic acid. After filtration and removal of the solvent, the residue was triturated with cold CH_2Cl_2 (2×) and ether (1×). The residue was then crystallized from hot $CHCl_3$ to give the hydroxamate (79%) (single component by ^1H NMR and TLC) as a white solid.

Scheme C. Coupling acetylene **7** with bromo ester **9**(Bz) gave, after purification (silica gel, hexane/ether, (19:1)), 6-TBDMSO-**2**(Bz) in 57% yield: MS, [M + 1]$^+$ 409; NMR 0.01 (3 H, s), 0.10 (3 H, s), 0.93 (9 H, s), 5.29 (1 H, d, J = 5.4 Hz), 5.97 (1 H, d, J = 15.4 Hz), 6.19 (1 H, dd, J = 14.9 Hz, 5.4 Hz), 6.44 (1 H, dd, J = 14.9 Hz, 11.1 Hz), 7.25–7.38 (11 H, m). However, in an attempt at simultaneous desilylation and hydrolysis of 6-TBDMSO-**2**(Bz)

Table III. Toxicity of Isobutylamide 1 and Its Candidate Metabolites on Injection into Mice and Houseflies

compd	LD_{50}, mg/kg mouse[a]	housefly[b]
1	3.2	0.33[c]
metabolites[d]	>8	>1.6

[a] Intraperitoneal administration. [b] Injected into the thorax following PB administration to the abdomen. [c] Topical LD_{50} values (mg/kg) are 17 alone and 0.8 with PB pretreatment. [d] The compounds individually tested were *p*-HO-1, 6-HO-1, 6-keto-1, β-HO-1, β,6-$(HO)_2$-1, 2, 6-HO-2, 3, 6-HO-3, and 4.

to the desired hydroxy acid with BBr_3, only a 22% yield of 6-HO-2 was obtained on purification (silica gel, ether/hexane (4:1)). In a similar manner, acetylene 7 was coupled with bromo ester 10(Me) and then purified (silica gel, hexane/ether (19:1)) to give 6-TBDMSO-2(Me) in 55% yield: MS, $[M + 1]^+$ 333; NMR 0.01 (3 H, s), 0.10 (3 H, s), 0.95 (9 H, s), 3.73 (3 H, s), 5.30 (1 H, d, J = 4.6 Hz), 5.93 (1 H, d, J = 15.3 Hz), 6.19 (1 H, dd, J = 15.0 Hz, 5.4 Hz), 6.45 (1 H, dd, J = 15.0 Hz, 11.2 Hz), 7.22–7.36 (6 H, m). This was then converted to the corresponding primary amide (6-TBDMSO-3) via the aluminum amide reagent derived from ammonium chloride, as described for the formation of 3 above. Purification (silica gel, ethyl acetate/hexane (6:1)) furnished the desired primary amide (6-TBDMSO-3) as a pale yellow oil: 62%; MS, $[M + 1]^+$ 318; NMR 0.01 (3 H, s), 0.10 (3 H, s), 0.94 (9 H, s), 5.28 (1 H, d, J = 5.4 Hz), 5.95 (1 H, d, J = 15.1 Hz), 6.14 (1 H, dd, J = 15.0 Hz, 5.4 Hz), 6.40 (1 H, dd, J = 15.0 Hz, 11.0 Hz), 7.18 (1 H, dd, J = 15.1 Hz, 11.0 Hz), 7.23–7.34 (5 H, m). Desilylation was achieved with tetrabutylammonium fluoride to give, after purification (silica gel, ethyl acetate), the desired product 6-HO-3 (95%).

β,6-$(HO)_2$-1 as the TBDMSO ether was prepared by coupling 6-TBDMSO-2(Me) with the aluminum amide reagent derived from 2-hydroxyisobutylamine protected as the TBDMSO ether (Basha et al., 1977). A 2.0 M solution of trimethylaluminum in toluene (0.34 mmol) was slowly added at room temperature to a solution of 2-TBDMSO-isobutylamine (0.3 mmol) in dry CH_2Cl_2 (1 mL) under nitrogen. The mixture was stirred at room temperature for 15 min and the ester 6-TBDMSO-2(Me) (0.27 mmol) was added in dry CH_2Cl_2 (0.5 mL). The mixture was warmed to 45 °C. After 20 h, the reaction was complete as judged by TLC analysis (hexane/ethyl acetate (4:1)). The reaction was carefully quenched by pouring into aqueous 1 M HCl, and then the mixture was extracted with CH_2Cl_2. The organic extract was dried and concentrated to give an orange oil. Purification (silica gel, hexane/ethyl acetate (9:1)) gave a colorless oil of β,6-$(TBDMSO)_2$-1: 78%; MS, $[M + 1]^+$ 504; NMR 0.03 (3 H, s), 0.12 (3 H, s), 0.16 (6 H, s), 0.94 (9 H, s), 0.97 (9 H, s), 1.27 (6 H, s), 3.32 (2 H, d, J = 5.8 Hz), 5.31 (1 H, d, J = 5.6 Hz), 5.82 (1 H, br t, J = 5.5 Hz), 5.90 (1 H, d, J = 15.0 Hz), 6.16 (1 H, dd, J = 15.0 Hz, 5.6 Hz), 6.42 (1 H, dd, J = 15.0 Hz, 11.1 Hz), 7.23 (1 H, dd, J = 15.0 Hz, 11.1 Hz), 7.27–7.38 (5 H, m). Desilylation with tetrabutylammonium fluoride in dry THF followed by purification (silica gel, ethyl acetate) furnished β,6-$(HO)_2$-1 as an oil (72 %).

SPECTROSCOPIC AND CHROMATOGRAPHIC PROPERTIES

Isobutylamide 1 and its candidate metabolites are identified by their NMR (Table I) and mass (Table II) spectral features. The compounds are readily separated by HPLC and GC (Table II) for metabolite analysis (Johnston et al., 1989).

Analysis of 4 can be accomplished by treatment with diazomethane (ether, −15 °C, 12 h) and GC–MS on the SPB5 system (Table II), giving three peaks assigned as dimethyl derivatives ($[M + 1]^+$ 232; R_t 8.6, 11.3 min (major) and 12.4 min (minor)) and four peaks apparently associated with monomethyl derivatives ($[M + 1]^+$ 218; R_t 9.7, 9.9, 10.1, 10.6 min). The major dimethyl derivatives are probably the *E* and *Z* isomers of the $-C(OCH_3)=NOCH_3$ compound, and the minor one is possibly the $-C(O)N(CH_3)OCH_3$ derivative (Beart and Ward, 1974).

BIOLOGICAL ACTIVITIES

The test compounds were administered intraperitoneally to male albino Swiss–Webster mice (18–22 g) with methoxytriglycol (10–100 μL) as the carrier vehicle for mortality determinations after 24 h. Alternatively, they were injected into the thorax of adult female houseflies (*Musca domestica* SCR susceptible strain) with 50% aqueous acetone (0.2 μL) as the carrier vehicle 1 h after topical treatment on the abdomen with 5 μg of piperonyl butoxide (PB) applied in 0.5 μL of acetone, again with mortality determination after 24 h. Compound 1 was also administered topically with application in 0.5 mL of acetone with or without PB pretreatment as above.

Isobutylamide 1 is quite toxic with injected LD_{50} values of 3.2 mg/kg for mice and 0.33 mg/kg for houseflies with PB pretreatment (Table III). It is also strongly synergized by PB on topical application to houseflies. None of the candidate metabolites is toxic to mice and houseflies at the highest doses injected, making them less than one-third as toxic as 1 to mice and PB-pretreated houseflies (Table III).

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LITERATURE CITED

Aihara, T. On the Principles of *Xanthoxylum piperitum* DC. IV. The Structure of Sanshoamide. *J. Pharm. Soc. Jpn.* **1951**, *71*, 1112–1115.

Aizpurua, J. M.; Palomo, C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU): an Effective Base for Introduction of *t*Butyldimethylsilyl Group in Organic Compounds. *Tetrahedron Lett.* **1985**, *26*, 475–476.

Barrelle, M.; Glenat, R. Synthese et Spectrographie IR et UV de Cétones α-Acétyléniques Vraies. *Bull. Soc. Chim. Fr.* **1967**, 453–458.

Basha, A.; Lipton, M.; Weinreb, S. M. A Mild, General Method for Conversion of Esters to Amides. *Tetrahedron Lett.* **1977**, 4171–4174.

Beart, P. M.; Ward, A. D. Hydroxamic Acids. V. The Alkylation of *O*-Alkylarylhydroxamic Acids. *Aust. J. Chem.* **1974**, *27*, 1341–1349.

Bhatt, M. V.; Kulkarni, S. U. Cleavage of Ethers. *Synthesis* **1983**, 249–282.

Blade, R. J., personal communication, 1987.

Blade, R. J.; Robinson, J. E. A Novel Application of Molybdenum Mediated Diene Synthesis in the Preparation of Piperovatine. *Tetrahedron Lett.* **1986**, 3209–3210.

Corbett, M. D.; Chipko, B. R. Quantitative Determination of *N*-Arylaceto- and *N*-Arylglycolhydroxamic Acids in Biochemical Reaction Mixtures. *Anal. Biochem.* **1979**, *98*, 169–177.

Crombie, L. The Structure of an Insecticidal Isobutylamide from Pellitory Root. *Chem. Ind. (London)* **1952**, 1034–1035.

Crombie, L.; Hobbs, A. J. W.; Horsham, M. A. Hydrozirconation Methods for Natural Isobutylamides (Anacyclin, Pellitorine and Its Vinylogue) and Synthons. *Tetrahedron Lett.* **1987**, *28*, 4875–4878.

DeJongh, D. C.; Lin, D. C. K.; LeClair-Lanteigne, P.; Gravel, D. Mass Spectrometry of *N*-Benzoyl-2-hydroxyalkylamines. Role of the Hydroxylic Hydrogen in the Fragmentation Pattern. *Can. J. Chem.* **1975**, *53*, 3175–3187.

Elliott, M. Lipophilic Insect Control Agents. In *Recent Advances in the Chemistry of Insect Control*; Janes, N., Ed.; Royal Society of Chemistry: London, 1985; pp 73–102.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A.; Sawicki, R. M. Insecticidal Amides with Selective Potency Against a Resistant (*Super-kdr*) Strain of Houseflies (*Musca domestica* L.). *Agric. Biol. Chem.* **1986**, *50*, 1347–1349.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 1: Introductory Survey, and Discovery of an Active Synthetic Compound. *Pestic. Sci.* **1987a**, *18*, 191–201.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 2: Effect of Heteroatom Replacements and of Introducing Methyl Groups in the Pentadiene System. *Pestic. Sci.* **1987b**, *18*, 203–209.

Jacobson, M. The Unsaturated Isobutylamides. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Dekker: New York, 1971; pp 137–176.

Johnston, J. J.; Horsham, M. A.; Class, T. J.; Casida, J. E. Metabolites of the Prototype Insecticide (2*E*,4*E*)-*N*-Isobutyl-6-phenylhexa-2,4-dienamide. 2. Formation in Mouse and Rat Liver Microsomal Systems, Rat Hepatocytes, and Houseflies. *J. Agric. Food Chem.* **1989**, following paper in this issue.

Jones, E. R. H.; Skatteböl, L.; Whiting, M. C. Researches on Acetylenic Compounds. Part LIV. The Preparation and Synthetical Applications of Ethynylmagnesium Bromide. *J. Chem. Soc.* **1956**, 4765–4768.

King, F. D.; Pike, S.; Walton, D. R. M. Silylhydroxylamines as Reagents for High-yield RCOCl → RNCO Conversions. *J. Chem. Soc., Chem. Commun.* **1978**, 351–352.

Levin, J. I.; Turos, E.; Weinreb, S. M. An Alternative Procedure for the Aluminum-Mediated Conversion of Esters to Amides. *Synth. Commun.* **1982**, *12*, 989–993.

McOmie, J. F. W.; Watts, M. L.; West, D. E. Demethylation of Aryl Methyl Ethers by Boron Tribromide. *Tetrahedron* **1968**, *24*, 2289–2292.

Mestres, R.; Palomo, C. A New Reagent for Activating Carboxy Groups; Preparation and Reactions of Phenyl *N*-Phenylphosphoramidochloridate. *Synthesis* **1982**, 288–291.

Miyakado, M.; Nakayama, I.; Yoshioka, H.; Nakatani, N. The *Piperaceae* Amides I: Structure of Pipercide, a New Insecticidal Amide from *Piper nigrum*. L. *Agric. Biol. Chem.* **1979**, *43*, 1609–1611.

Miyakado, M.; Nakayama, I.; Ohno, N.; Yoshioka, H. Structure, Chemistry and Actions of the Piperaceae Amides: New Insecticidal Constituents Isolated from the Pepper Plant. In *Natural Products for Innovative Pest Management*; Whitehead, D. L., Bowers, W. S., Eds.; Pergamon: New York, 1983; pp 369–382.

Mulvaney, J. E.; Folk, T. L.; Newton, D. J. Polylithiated Species from 1-Phenylpropyne and Organolithium Reagents. *J. Org. Chem.* **1967**, *32*, 1674–1675.

Osborne, N. F. The Chemistry of 4-Mercaptoazetidin-2-ones. Part 2. Synthesis of Bisnorpenicillins. *J. Chem. Soc., Perkin Trans. 1* **1980**, 150–155.

Pincock, J. A.; Somawardhana, C. The Bromination of 3-Arylpropynes: Evidence for Phenyl Participation at a Vinyl Cation. *Can. J. Chem.* **1978**, *56*, 1164–1169.

Price, S. J.; Pinder, A. R. Chemistry of *Ottonia vahlii* Kth. II. Constitution of the Nonvolatile Component. *J. Org. Chem.* **1970**, *35*, 2568–2570.

Su, H. C. F. *N*-Isobutylamides. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*; Kerkut, G. A., Gilbert, L. I., Eds.; Pergamon: New York, 1985; Vol. 12, pp 273–289.

Winterfeldt, E. Strukturaufklärung und Synthese einer Thiophenverbindung aus *Chrysanthemum frutescens* L. *Chem. Ber.* **1963**, *96*, 3349–3358.

Yates, P.; Auksi, H. The Synthesis of Bicyclo[2.2.2]octenones via Intramolecular Diels-Alder Reactions of Modified Wessely Oxidation Products. *Can. J. Chem.* **1979**, *57*, 2853–2863.

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Metabolites of the Prototype Insecticide (2*E*,4*E*)-*N*-Isobutyl-6-phenylhexa-2,4-dienamide. 2. Formation in Mouse and Rat Liver Microsomal Systems, Rat Hepatocytes, and Houseflies

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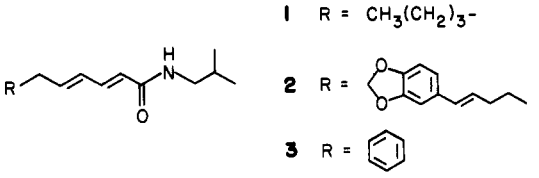
The metabolism of (2*E*,4*E*)-*N*-isobutyl-6-phenylhexa-2,4-dienamide is examined as a prototype of the natural and synthetic isobutylamide insecticides. Nine metabolites from mouse and rat liver microsomal systems, rat hepatocytes, and/or houseflies are identified by HPLC, GC, and GC–MS comparisons with synthetic standards. The parent isobutylamide yields the corresponding unsubstituted amide on *N*-methylene hydroxylation in the microsomal oxygenase system. Both of these amides are readily hydrolyzed by rat but not mouse amidases. The unsubstituted amide in mouse microsomes appears to undergo sequential enzymatic oxidation and hydrolysis to the corresponding carboxylic acid; the presumed hydroxamic acid intermediate is not detected. Additional metabolites are the β-hydroxyisobutyl, 6-hydroxy, 6-keto, and *p*-hydroxy derivatives of the parent isobutylamide and the 6-hydroxy derivatives of the *N*-(β-hydroxyisobutyl) compound and of the unsubstituted amide and carboxylic acid. Hepatocytes conjugate some of these metabolites. The persistence and toxicity of this prototype insecticide are limited by oxidative metabolism at multiple sites in the isobutyl and benzyl moieties.

More than 20 insecticidal isobutylamides [*N*-(2-methylpropyl)amides] are identified from plants of the families Piperaceae, Compositae, and Rutaceae (Jacobson, 1971; Elliott, 1985; Su, 1985; Elliott et al., 1987). They include the *N*-isobutyl-2,4-dienamides pellitorine (**1**) and pipercide (**2**) (one of the isobutylamide constituents of dietary black pepper) (Crombie, 1952; Su and Horvat, 1981; Miyakado et al, 1983; Crombie and Denman, 1984).



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