JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Synthesis and Insecticidal Activity of Spinosyns with C9-*O*-Benzyl Bioisosteres in Place of the 2′,3′,4′-Tri-*O*-methyl Rhamnose

M. Paige Oliver, Gary D. Crouse, David A. Demeter, and Thomas C. Sparks*

Dow AgroSciences, Discovery Research, 9330 Zionsville Road, Indianapolis, Indiana 46268, United States

Supporting Information

ABSTRACT: The spinosyns are fermentation-derived natural products active against a wide range of insect pests. They are structurally complex, consisting of two sugars (forosamine and rhamnose) coupled to a macrocyclic tetracycle. Removal of the rhamnose sugar results in a >100-fold reduction in insecticidal activity. C9-*O*-benzyl analogues of spinosyn D were synthesized to determine if the 2′,3′,4′-tri-*O*-methyl rhamnose moiety could be replaced with a simpler, synthetic bioisostere. Insecticidal activity was evaluated against larvae of *Spodoptera exigua* (beet armyworm) and *Helicoverpa zea* (corn earworm). Whereas most analogues were far less active than spinosyn D, a few of the C9-*O*-benzyl analogues, such as 4-CN, 4-Cl, 2-isopropyl, and 3,5-diOMe, were within 3−15 times the activity of spinosyn D for larvae of *S. exigua* and *H. zea*. Thus, although not yet quite as effective, synthetic bioisosteres can substitute for the naturally occurring 2′,3′,4′-tri-*O*-methyl rhamnose moiety.

KEYWORDS: *macrocyclic lactone insecticides, spinosyns, semisynthetic analogues, natural products, rhamnose sugar bioisostere*

INTRODUCTION

The global demand for food continues to expand in response to a rapidly growing world population.[1,2] As important competitors for food crops,[3] the control of pest insects remains an ongoing, essential component to successful food production. However, pest insect resistance to existing insecticides continues to increase,[4−6] limiting the utility of many established classes of chemicals and creating the need for new pest insect control options. Simultaneously, regulatory requirements and costs for developing and registering new pesticides continue to rise,[7,8] making the discovery and development of new insect control agents a substantial challenge.

In the quest for new insect control agents, natural products have been and remain an excellent source of novel chemistry and inspiration for insecticides.[9,10] Although very few natural products are currently in extensive use as pesticides, based on global sales,[10] they have served as templates and inspiration for a wide range of fungicides, herbicides, and fungicides.[9−12] Among natural product-based insecticides, the spinosyns are a unique family of fermentation-derived, large, complex macrocyclic lactones possessing a novel mode of action.[13,14] As exemplified by the first spinosyn-based product, spinosad (Figure 1), the spinosyns are highly effective as insect control agents.[13,15,16] Continued exploration of the spinosyns through modifications of the rhamnose sugar led to semisynthetic spinosyn derivatives with greater insecticidal efficacy and spectrum giving rise to a second commercial insecticide, spinetoram[16,17] (Figure 1). Similarly, modifications to the sugar moieties of the avermectins resulted in significant shifts in spectrum and efficacy (e.g., emamectin benzoate).[18,19] Several studies have investigated the replacement of one or both of the oleandrose sugars of the avermectins with nonsugar bioisosteres resulting in insecticidal analogues.[20−23] Similarly, the milbemycins are insecticidal−acaricidal non-sugar-containing analogues of avermectin.[18] The addition of a nonsugar bioisostere to a milbemycin derivative resulted in a new lepidopteran active insecticide, lepimectin.[21]

The unique nature of the spinosyns and the appearance of resistance in some insect pests[24] have prompted further synthetic exploration of the spinosyn structure with the goal of simplifying the spinosyn tetracycle and producing analogues that potentially circumvent mechanisms of spinosyn resistance.[25,26] Other simplifications of the spinosyn structure potentially leading to improvement to insecticidal activity and/or spectrum could arise by replacement of the naturally occurring sugars with synthetic moieties. As demonstrated by the research leading to spinetoram, modifications of the rhamnose sugar can lead to increased insecticidal efficacy.[13,17] A previous study[27] examined the insecticidal efficacy of replacing rhamnose with alternative sugars and a few selected nonsugar moieties. Although some of the alternative sugars proved to be insecticidal, spinosyn analogues coupled with 9-*O*-(substituted) benzoyl or other nonsugar moieties as rhamnose replacement bioisosteres were insecticidally inactive against larvae of *Heliothis virescens* (tobacco budworm).[27] Spinosyn analogues containing a C9-*O*-(arylalkyl) oxime group (Figure 2) have also been reported to be insecticidal, although these analogues also contained either a 17-β-D-desosaminyl sugar or a 17-hydroxy group instead of a forosamine sugar.[28] Because the nonsugar bioisosteres examined were limited in number, the objective of the present study is to extend the examination of nonsugar rhamnose bioisosteres to determine whether simple *O*-benzyl substitutions (Figure 2) would be more suitable bioisosteric replacements for the spinosyn rhamnose sugar.

Received: January 2, 2015
Revised: May 19, 2015
Accepted: May 20, 2015
Published: May 20, 2015

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577

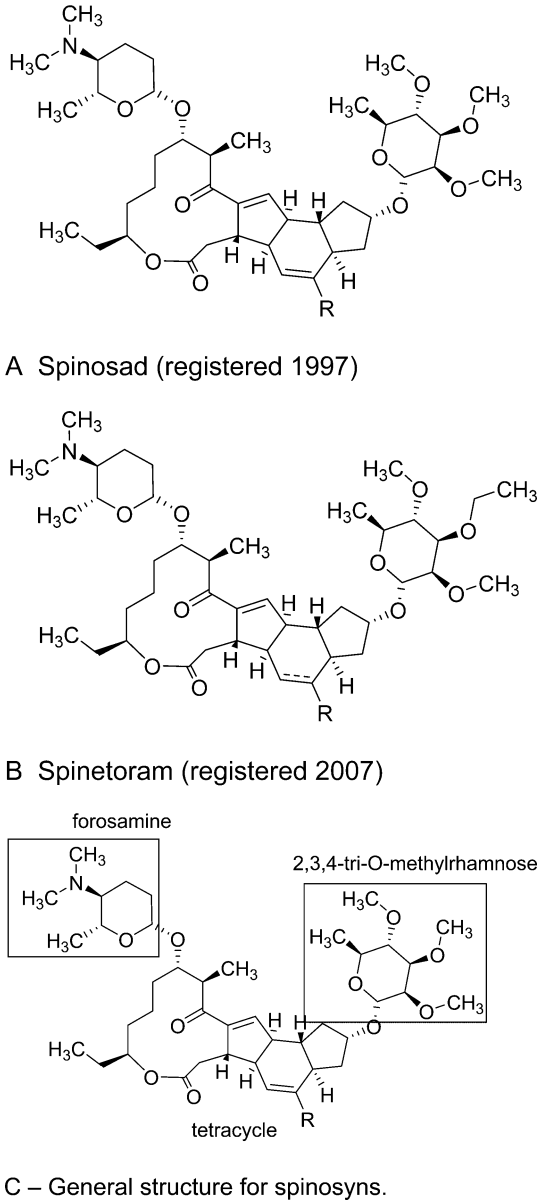

Figure 1. Structures of spinosad, spinetoram, spinosyns: (A) spinosad (primary component spinosyn A (R = H), minor component spinosyn D (R = Me)); (B) spinetoram (primary component 5,6-dihydro-3′-*O*-ethyl spinosyn J (R = H, 5,6 single bond), minor component 3′-*O*-ethyl spinosyn L (R = Me, 5,6-double bond)); (C) general structure of the spinosyns (R = H or Me, boxes highlight the two sugar moieties).

MATERIALS AND METHODS

Synthesis of Spinosyn Analogues. Unless otherwise indicated, all commercial reagents were used without purification. Solvents were of reagent grade. Benzyl alcohol precursors were available commercially or were prepared using published routes. Purity was established as >95% by ^{1}H NMR spectroscopy and LC-MS. Mass spectral data were obtained by electron ionization on one of several systems including a series 1100 mass selective detector (MSD) (Hewlett-Packard, Palo Alto, CA, USA) or a 5890 series II (Hewlett-Packard) with a 5890A mass spectrometer (Hewlett-Packard) or a 6890 series GC system with a 5973 MSD (Hewlett-Packard). Mass spectral data were also obtained by LC-MS analysis using a 215 liquid handler (Gilson, Middleton, WI, USA) for injection coupled to a 1100 chromatography system (Agilent Technologies, Santa Clara, CA, USA) composed of a quaternary pump system and photodiode array detector linked to a 50/50 splitter going simultaneously to a 2000 evaporative light scattering detector (Alltech Associates, Inc., Deerfield, IL, USA) with impactor on and to a ZQ mass spectrometer detector (Waters Corp., Milford, MA, USA) or by a second LC-MS (Waters Corp.) composed of a 2777 autosampler, 1525 μL binary pumps with 100 μL pump heads, and a 2996 photodiode array detector linked to a 50/50 splitter going simultaneously to a 2420 evaporative light scattering detector (Alltech Associates, Inc.) and a ZQ mass spectrometer detector (Waters Corp.). Masses are detected by electrospray ionization (ESI) on both systems. The main method consists of a linear gradient from 5 to 95% organic in 5 min. Solvents are 94.9% H_2O with 5% acetonitrile (aqueous) and 99.9% acetonitrile (organic) both spiked with 0.1% acetic acid. The column used was a 4.6 mm × 50 mm i.d., 5 μm, Sunfire Prep C18 OBD (Waters Corp.). Reverse phase high-performance liquid chromatography (RP HPLC) was conducted on a liquid handler 215 (Gilson) with HPLC grade acetonitrile and water (both with 0.1% acetic acid). ^{1}H NMR spectroscopy data were collected using chloroform-*d* solvent with tetramethylsilane as internal standard.

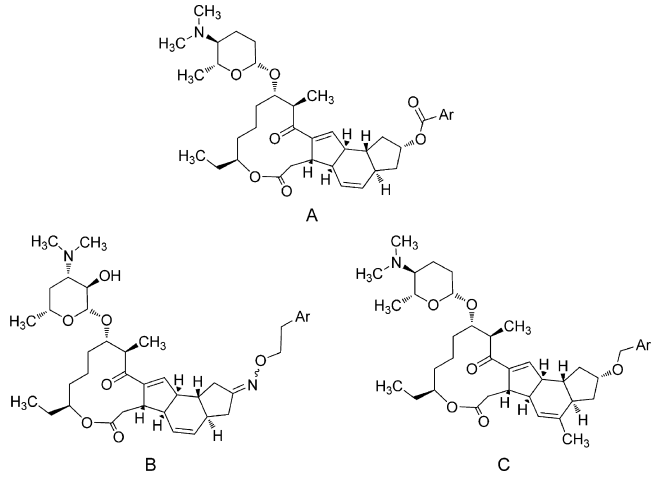

Figure 2. Spinosyn analogues containing rhamnose replacements at C-9: (A) 9-*O*-benzoyl analogues; (B) 9-(*O*-aralkyl) oxime analogues; (C) 9-*O*-benzyl analogues.

Preparation of 3,4,5-Trimethoxybenzyl Bromide. In a 1 dram vial equipped with a magnetic stir bar were added 0.198 g of 3,4,5-trimethoxybenzyl alcohol (1.00 mmol), 1.5 mL of 9:1 dichloromethane/diethyl ether, and hydrogen bromide (40% aqueous solution). The reaction mixture was stirred for 4.5 h. The reaction mixture was poured into a 25 mL vial containing water and ice. This mixture was extracted three times with ethyl acetate, dried over magnesium sulfate, filtered, concentrated under reduced pressure and low heat, and then stored in the freezer.

Preparation of 9-*O*-Benzyl Spinosyn D Analogues. *Method A: Preparation of 9-O-(3,4,5-Tri-O-methylbenzyl)spinosyn D,* ***6q.*** In a 1 dram vial equipped with a magnetic stir bar were added 0.144 g of 3,4,5-trimethoxybenzyl bromide (0.55 mmol), 0.10 g (0.18 mmol) of spinosyn L C-9 pseudoaglycone, 0.150 g of powdered 10:1 potassium hydroxide/tertrabutylammonium hydride (excess), and 3.0 mL of dichloromethane. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether and then passed through a 10 mL Celite cartridge. The eluent was concentrated under vacuum. Purification using a Redi Sep 10 g flash column (Isco, Inc., Lincoln, NE, USA), eluting with ethyl acetate/hexanes/dichloromethane/methanol (10:10:10:1, v/v/v/v), yielded 0.066 g (50% yield) of **6q** as a solid foam.

Method B: Preparation of 9-O-(2-Isopropylbenzyl)spinosyn D, ***6d.*** In a 1 dram vial equipped with a magnetic stir bar were added 0.050 g of 1-(bromomethyl)-2-isopropylbenzene (0.21 mmol), 0.020 g (0.036 mmol) of spinosyn L C-9 pseudoaglycone, 0.10 g of powdered 10:1 potassium hydroxide/tertrabutylammonium hydride (excess), and 1.5 mL of dichloromethane. The reaction mixture was stirred overnight at room temperature. The supernatant solution was then

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577

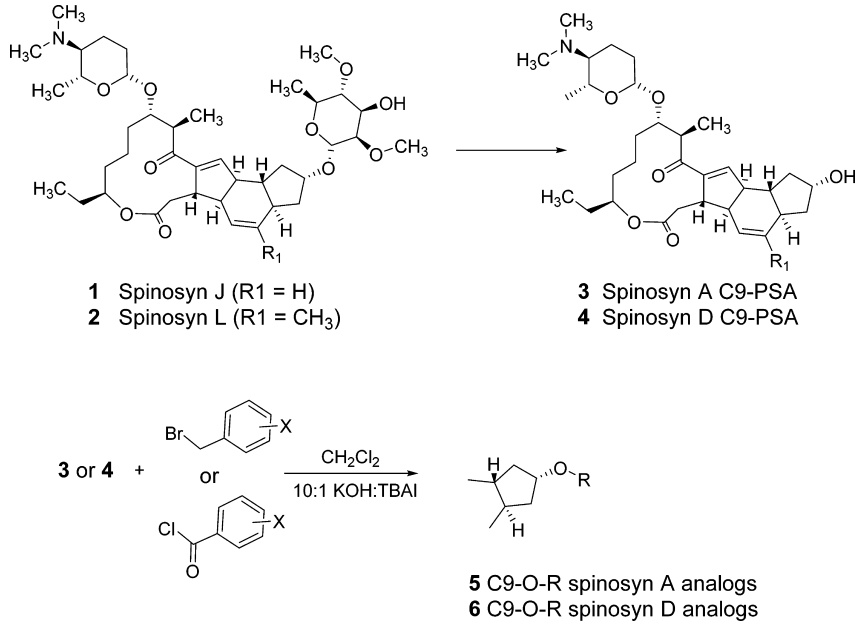

Figure 3. Synthesis of spinosyn D C9 pseudoaglycone and spinosyn D analogues with rhamnose replacements.

added directly to the top of a Redi Sep 2.5 g flash column (Isco, Inc.) and eluted, first with 25 mL of dichloromethane, then with 25 mL of 1% methanol in dichloromethane, and finally with 25 mL of 2% methanol in dichloromethane, which resulted in elution of the desired 9-*O*-benzyl product. Removal of solvent furnished 0.0205 g (79%) of **6d** as a solid foam.

Molecular Modeling Overlays. The spinosyn D and the benzyl and benzoyl structures were built by hand in Sybyl-X 2.1 (Certata, Princeton, NJ, USA) starting from the minimized X-ray structure of spinosyn A. The structures were then minimized and flexibly fit to the minimized X-ray structure of spinosyn A. All of the molecules were initially geometry optimized using the Tripos force field[29] and Gasteiger–Huckel charges[30,31] (Tripos Associates, St. Louis, MO, USA). The shape-based flexible fit of spinosyn D and the benzyl and benzoyl structures was done using Surflex-Sim v2.706 distributed with Sybyl-X 2.1. The top-scoring conformer of each compound from Surflex-Sim was used in the overlay.

Insecticide Bioassays. Dose–response data for each compound against two susceptible laboratory strains of the lepidopteran species *Spodoptera exigua* or *Helicoverpa zea* was determined with a diet-based bioassay using 128-well diet trays (Bio-Serv, Frenchtown, NJ, USA). Three to five second-instar larvae of either lepidopteran were placed in each well (3 mL) of the diet tray that had been previously filled with 1 mL of artificial diet. Selected dosages (12.5, 3.125, 0.78, 0.195, 0.049 μg/cm^2) of the test compound (dissolved in 50 μL of 90:10 acetone/water mixture, v/v) were then applied to the diet in each of 16 wells, allowed to dry, and covered with a clear self-adhesive cover. Controls received solvent only. All treatments were held at 25 °C and 14:10 h light/dark for 6 days. Each well was recorded as a unit (all alive or all dead), the activity across the 16 wells (for each dose) was then averaged. The lethal concentration for 50% of the test population (LC_{50}) and 95% fiducial limits for each compound, with correction for mortality in the controls, were determined using probit analysis.[32]

Bioassays against a susceptible laboratory strain of *Aphis gossypii* (cotton aphid) were conducted as described previously.[33] Briefly, 1-week-old summer crookneck squash seedlings, *Cucurbita pepo*, were pruned to a single cotyledon. A mixed population (immatures and adults) of *A. gossypii* was transferred to the cotyledons 16–24 h prior to the application of test materials. Test compounds were dissolved in 90:10 acetone/ethanol, v/v, to form a stock solution, from which an appropriate amount was diluted in water containing 0.05% Tween 20 to form the spray solution of 50 and 200 ppm. Application was made using a hand-held DeVilbiss airbrush sprayer on aphid-infested plants spraying both leaf surfaces until runoff. There were four replicates (plants) for each treatment, whereas controls consisted of eight replicates treated with a solvent blank. Following treatment, plants were held at 23 °C and 40% RH with a 24 h photoperiod prior to grading. Grading occurred at 72 h post-treatment and consisted of live aphid counts (all nonwinged stages) on each of the replicates compared to the averaged aphid population on the solvent blank controls to estimate a percent mortality.

RESULTS AND DISCUSSION

Synthesis. Rhamnose replacement analogues were made by first deglycosylating spinosyns J, **1**, and L, **2** (Figure 3), to form the pseudoaglycones **3** and **4**, respectively, as described previously.[34] A free C-9 hydroxyl group was then available to prepare benzylated or benzoylated derivatives. Twenty-eight benzyl bromides were selected on the basis of diverse steric and electronic properties. Some of these were available commercially, with the remainder prepared as shown in Figure 3. Commercially available benzyl alcohols were converted into the bromides using hydrogen bromide in dichloromethane/ether. When the benzyl alcohols themselves were unavailable, they were prepared either from the corresponding aldehyde, for **6x**, or acid, for **6l**, by reduction with sodium borohydride or lithium aluminum hydride ($LiAlH_4$), respectively. The substituted aryl methyl bromides were then reacted with **3** or **4**, using individual 1 dram vials with powdered potassium hydroxide/tetra-*n*-butylammonium iodide as the basic catalyst to produce the desired targets **5** and **6** (Figure 3). In addition to exhibiting the expected mass parent ion by LC-MS, the 9-*O*-benzyl products were also characterized by the presence of a $-OC\underline{H}_2-$ signal in the proton NMR between 4.4 and 4.56 ppm, in addition to the expected aromatic signals. This methylene peak was usually a singlet; however, due to its diastereotopic nature, it was sometimes observed as a doublet of doublets. Isolated yields were usually in the range of 50–85%, except when highly electron-rich benzyl bromides such as 2,4-dimethoxy, 2-methoxy, and 4-methoxy were used; in these cases, quaternization of the tertiary amine predominated under the conditions used for this reaction. Structures and selected

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571–5577

physical property data of all final targets are provided in Table 1.

Table 1. Structure of 9-*O*-Benzyl or -*O*-Benzoyl Spinosyn Analogues

analogue	linker	phenyl substitution	MS (ESI) $[M + H]^+$
5a	−CO−	3-OCH_3	678
5b	−CH_2−	3-OCH_3	664
6a	−CO−	3-OCH_3	692
6b	−CH_2−	3-OCH_3	678
6c	−CH_2−	unsubstituted	648
6d	−CH_2−	2-iPr	690
6e	−CH_2−	2-$OCH_2CH_2OCH_3$	722
6f	−CH_2−	2,3-$(CH_3)_2$	676
6g	−CH_2−	2,4-$(CH_3)_2$	676
6h	-CH_2-	2-OCH_3, 4-CO_2CH_3	736
6i	−CH_2−	2-OCH_3, 4-Cl	712
6j	−CH_2−	2-F, 4-CN	691
6k	−CH_2−	2-Cl, 6-CH_3	696
6l	−CH_2−	2,6-$(CH_3)_2$	676
6m	−CH_2−	3-OCF_3	732
6n	−CH_2−	3-$CH_2OC_2H_5$	706
6o	−CH_2−	3-CF_3, 4-OCH_3	746
6p	−CH_2−	3,5-$(OCH_3)_2$	708
6q	−CH_2−	3,4,5-$(OCH_3)_3$	738
6r	−CH_2−	4-Cl	682
6s	−CH_2−	4-CN	673
6t	−CH_2−	4-CH_3	662
6u	−CH_2−	4-CF_3	716
6v	−CH_2−	4-iPr	690
6w	−CH_2−	6-fluoro-4*H*-benzo[1,3]dioxin-8-yl	724
6x	−CH_2−	benzofuran-2-yl	688

Insecticidal Activity. Earlier work using C9-benzoyl derivatives had evaluated 2-, 3-, and 4-methoxy benzoyl analogues,[27] as well as a C9-*O*-methyl. Because of the high reactivity of *o*- and *p*-methoxy-substituted benzyl bromides, which led to the formation of quaternary salts under our reaction conditions, the initial study focused on 3-methoxy analogues of spinosyns A and D, **5b** and **6b**, respectively (Table 2). These compounds were evaluated for insecticidal efficacy against two lepidopteran species, *S. exigua* and *H. zea*. As shown in Table 2, the C9-*O*-(3-methoxybenzyl) derivatives of spinosyns A and D, **5b** and **6b**, were more active than the corresponding C9 pseudoaglycones or the respective C9-*O*-benzoyl derivatives, **5a** and **6a**. However, neither **5b** nor **6b** was as potent as spinosyn A or D (Table 2).

Molecular Modeling Overlay. The 3-methoxybenzoyl, **6a**, and 3-methoxybenzyl, **6b**, derivatives of spinosyn D were overlaid on the structure of spinosyn D. As shown in Figure 4,

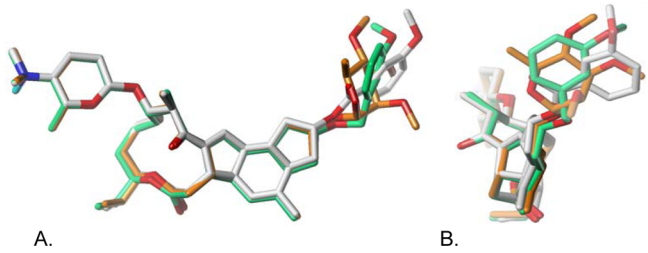

Figure 4. Overlays of C9 3-*O*-methylbenzyl, **6a** (green), and 3-*O*-methylbenzoyl, **6b** (light gray), spinosyn D derivatives, with spinosyn D (orange): (A) side view; (B) end-on view with the C9-benzyl and benzoyl bioisosteres in the foreground.

the benzyl derivative, **6b**, fits into the space of the rhamnose sugar better than the benzoyl derivative, **6a**. The better fit of the benzyl derivatives into the space of the rhamnose (Figure 4) may partially explain their improved insecticidal activity compared to the corresponding benzoyl derivatives (Table 2).

The above results prompted a more comprehensive evaluation of C9-*O*-benzyl analogues. For this study, spinosyn D analogues were prepared due to the availability of gram quantities of the corresponding 9-OH precursor. As noted in Table 2, the relative potencies of A and D analogues are similar. Although the C9-*O*-benzyl analogues displayed a wide range of insecticidal activity (Table 3), all of the analogues were less potent than spinosyn D. A few of the substitutions exhibited insecticidal activity against *S. exigua* that was within 5−15 times that of spinosyn D, whereas most were 20−200-fold less active than spinosyn D. The 2-isopropyl analogue, **6d**, was the most potent against *S. exigua* followed closely by the 3-*O*-methylethoxy, **6n**, 4-methyl, **6t**, 3,5-dimethoxy, **6p**, and benzofuran-2-yl, **6x**, analogues. For larvae of *H. zea*, the most active substitution was only about 3-fold less active than spinosyn D, with the majority being 20− 100-fold less active against *H. zea* than spinosyn D. The most effective substitution against larvae of *H. zea* was the 2-isopropyl, **6d**, followed closely by the 3,5-dimethoxy, **6p**, 4-Cl, **6r**, and 4-CN, **6s** (Table 3). All of the C9-*O*-benzyl analogues were also evaluated against *A. gossypii*. However, no insecticidal activity was observed against *A. gossypii* for any of the analogues at either the 50 or 200 ppm dose (data not shown).

Modifications of the spinosyn structure can greatly alter the insecticidal activity with significant improvements possible over the naturally occurring spinosyns.[13,17,35,36] As such, there has

Table 2. Insecticidal Activity of 9-*O*-Benzyl and 9-*O*-Benzoyl Spinosyns

compound	C9-O substitution	LC_{50}, $\mu g/cm^2$ (95% FL)	
		S. exigua	*H. zea*
spinosyn A	(2′,3′,4′-$(CH_3O)_3$ β-D-rhamnosyl)	0.20 (0.11−0.36)	0.20 (0.18−0.21)
spinosyn D	(2′,3′,4′-$(CH_3O)_3$ β-D-rhamnosyl)	0.044 (0.029−0.049)	0.11 (0.09−0.14)
spinA C9-PsA	H	>12.5	>12.5
spinD C9-PsA	H	7.9 (6.1−12.2)	12.5 (8.6−107)
5a	3-methoxybenzoyl A	4.8 (3.7−9.4)	6.3 (3.6−10.8)
5b	3-methoxybenzyl A	2.0 (1.0−2.7)	4.2 (3.1−8.1)
6a	3-methoxybenzoyl D	>12.5	>12.5
6b	3-methoxybenzyl D	1.4 (0.97−2.0)	5.3 (3.9−10.0)

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577

Table 3. Insecticidal Activity of 9-*O*-Benzyl Spinosyns

compound	C9- phenyl substitution	LC_{50}, $\mu g/cm^2$ (95% FL)	
		S. exigua	*H. zea*
spinosyn D		0.044 (0.029−0.049)	0.11 (0.09−0.14)
6b	3-OCH_3	1.4 (0.97−2.0)	5.3 (3.9−10.0)
6c	unsubstituted	1.6 (1.1−2.3)	1.8 (1.2−2.6)
6d	2-isopropyl	0.2 (0.0003−0.4)	0.3 (0.2−0.6)
6e	2-$OCH_2CH_2OCH_3$	1.7 (1.2−2.4)	10.7 (5.8−16.3)
6f	2,3-$(CH_3)_2$	1.3 (0.9−1.9)	8.2 (8.6−11.2)
6g	2,4-$(CH_3)_2$	1.6 (0.9−1.7)	3.9 (2.8−7.6)
6h	2-OCH_3, 4-CO_2CH_3	3.7 (2.7−7.1)	5.9 (2.09−851)
6i	2-OCH_3, 4-Cl	0.9 (0.5−1.5)	0.9 (0.5−1.5)
6j	2-F, 4-CN	0.9 (0.6−2.8)	1.6 (0.6−3.9)
6k	2-Cl, 6-CH_3	8.5 (4.3−10.7)	6.3 (4.5−9.3)
6l	2,6-$(CH_3)_2$	0.9 (0.5−1.5)	1.2 (0.7−3.4)
6m	3-OCF_3	13.0 (9.4−20.7)	9.6 (5.1−12.2)
6n	3-$OCH_2OC_2H_5$	0.3 (0.01−1.2)	∼17
6o	3-CF_3, 4-OCH_3	1.2 (0.8−1.8)	1.4 (0.8−2.1)
6p	3,5-$(OCH_3)_2$	0.5 (0.3−0.7)	0.5 (0.4−0.7)
6q	3,4,5-$(OCH_3)_3$	8.2 (5.5−12.4)	6.3 (3.6−12.8)
6r	4-Cl	0.7 (0.5−1.1)	0.6 (0.4−0.9)
6s	4-CN	0.7 (0.5−1.2)	0.6 (0.4−0.8)
6t	4-CH_3	0.4 (0.3−0.6)	4.3 (2.3−10.0)
6u	4-CF_3	0.8 (0.4−1.7)	2.4 (0.8−3.9)
6v	4-isopropyl	5.3 (2.7−8.0)	11.1 (7.2−15.5)
6w	6-fluoro-4*H*-benzo[1,3]dioxin-8-yl	0.6 (0.3−0.8)	3.9 (1.8−10.3)
6x	benzofuran-2-yl	0.4 (0.2−12.2)	2.0 (1.4−2.9)
6y	7-(OCH_3)-2-naphthyl	1.4 (0.7−1.7)	1.4 (1.0−2.0)

been interest in exploring further alterations[27,28,33,34,37−41] and especially simplification of some components or the core macrolides tetracycle as a potential means for further exploration of these novel, complex, macrolides.[13,38,41,42] As noted in prior studies, deviation from the 2′,3′,4′-tri-*O*-methyl-*β*-L-rhamnose, whether by removing it, altering the stereochemistry, removing one or more *O*-alkyl groups, or replacing it with D- sugars, furanose sugars, or nonsugars,[13,27,35,36] all result in a large reduction (usually >100-fold) in insecticidal activity. Interestingly, the C9-*O*-benzyl ethers evaluated in the present study retained a significant level of insecticidal potency, demonstrating that, at least to some degree, a substituted benzylic group is better able to mimic the size and placement of the tri-*O*-rhamnosyl group. However, efforts to more closely mimic the shape or electronics of the rhamnose through incorporation of methoxy substituents met with limited success. For example, although the 3,5-dimethoxybenzyl substitution, **6p**, was among the most potent analogues against *S. exigua* larvae, several other simple substitutions, including 4-CN, **6s**, 4-Me, **6t**, 2-isopropyl, **6d**, 4-Cl, **6r**, and 3-methoxyethoxy, **6n**, were equally or more active. Two of the larger moieties, the benzofuran-2-yl, **6x**, and 6-fluoro-4*H*-benzo[1,3]dioxin-8-yl, **6w**, were also insecticidal against *S. exigua* larvae (Table 3), suggesting that to some degree larger moieties can be tolerated as replacements for the 2′,3′,4′-tri-*O*-methyl rhamnose. In general, the analogues showing the greatest potency against *S. exigua* were also among the most active against *H. zea* larvae, with the exception of the larger analogues, **6x** and **6w**.

In contrast to their excellent lepidopteran insecticidal activity, the spinosyns typically are weak against many sap-feeding insects such as aphids.[13,16] For example, spinosyn D is only weakly active (LC_{50} = 50 ppm) against *A. gossypii* compared to commercial aphicides such as imidacloprid (0.06 ppm).[14] None of the C9-*O*-benzyl analogues exhibited any activity against this aphid at the highest dose tested (200 ppm). Thus, the rhamnose bioisosteres were unable to improve on the activity of the spinosyns toward aphids relative to spinosyn D.

Although none of the rhamnose bioisostere-based spinosyns in the present study were as insecticidally active as spinosyn D, several analogues were within 3−15 times, depending on the insect species. Thus, the analogues exhibiting insecticidal activity close to that of spinosyn D demonstrate that an *O*-benzyl moiety can be a reasonable bioisostere for the 2′,3′,4′-tri-*O*-methyl rhamnose. Therefore, as demonstrated by the present and previous studies, the replacement of the sugars on macrolides such as the avermectins, and potentially the spinosyns, with nonsugar bioisoteres is further demonstrated to be a viable approach to developing new insecticidal chemistries.

■ ASSOCIATED CONTENT

Supporting Information

Detailed information on the synthesis of the new spinosyn analogues in this study and supporting NMR data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b01987.

■ AUTHOR INFORMATION

Corresponding Author

*(T.C.S.) Phone: (317) 337-3064. Fax: (317) 337-3205. E-mail: tcsparks@dow.com.

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577

■ ACKNOWLEDGMENTS

We thank Cathy Young and James Gifford (Dow AgroSciences) for assistance with the bioassays, Ricky Hunter (Dow AgroSciences) for the insect photograph, and the journal's anonymous reviewers for their very helpful suggestions.

■ ABBREVIATIONS USED

FL, fiducial limits; RH, relative humidity; TBAI, tetra-*n*-butylammonium iodide

■ REFERENCES

(1) Godfray, H. C. J.; Beddington, J. R.; Crute, I. R.; Haddad, L.; Lawrence, D.; Muir, J. F.; Pretty, J.; Robinson, S.; Thomas, S. M.; Toulmin, C. Food security: the challenge of feeding 9 billion people. *Science* **2010**, *327*, 812−818.

(2) Tilman, D.; Balzer, C.; Hill, J.; Befort, B. L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 20260−20264.

(3) Popp, J.; Peto, K.; Nagy, J. Pesticide productivity and food security, a review. *Agron. Sustainable Dev.* **2013**, *33*, 243−255.

(4) Whalon, M. E.; Mota-Sanchez, D.; Hollingworth, R. M. Analysis of global pesticide resistance in arthropods. In *Global Pesticide Resistance in Arthropods*; Whalon, M. E., Mota-Sanchez, D., Hollingworth, R. M., Eds.; CAB International: Cambridge, MA, USA, 2008; pp 5−31.

(5) Tabashnik, B. E.; Mota-Sanchez, D.; Whalon, M. E.; Hollingworth, R. M.; Carriere, Y. Defining terms for proactive management of resistance to Bt crops and pesticides. *J. Econ. Entomol.* **2014**, *107*, 496−507.

(6) Sparks, T. C.; Nauen, R. IRAC: Mode of action classification and insecticide resistance management. *Pestic. Biochem. Physiol.* **2015**, *121*, 122−128.

(7) Lamberth, C.; Jeanmart, S.; Luksch, T.; Plant, A. Current challenges and trends in the discovery of agrochemicals. *Science* **2013**, *341*, 742−746.

(8) Sparks, T. C. Insecticide discovery: an evaluation and analysis. *Pestic. Biochem. Physiol.* **2013**, *107*, 8−17.

(9) Cantrell, C. L.; Dayan, F. E.; Duke, S. O. Natural products as sources for new pesticides. *J. Nat. Prod.* **2012**, *75*, 1231−1242.

(10) Gerwick, B. C.; Sparks, T. C. Natural products for pest control: an analysis of their role, value and future. *Pest Manage. Sci.* **2014**, *70*, 1169−1185.

(11) Godfrey, C. R. A., Ed. *Agrochemicals from Natural Products*; Dekker: New York, 1995.

(12) Rimando, A. M.; Duke, S. O. Natural products for pest management. In *Natural Products for Pest Management*; American Chemical Society: Washington, DC, USA, 2006; pp 2−21.

(13) Salgado, V. L.; Sparks, T. C. The spinosyns: chemistry, biochemistry, mode of action, and resistance. In *Insect Control: Biological and Synthetic Agents*; Gilbert, L. I., Gill, S. S., Eds.; Academic Press: New York, 2010; pp 207−243.

(14) Geng, C.; Watson, G. B.; Sparks, T. C. Nicotinic acetylcholine receptors as spinosyn targets for insect pest management. In *Target Receptors in the Control of Insect Pests: Part I, Advances in Insect Physiology*; Cohen, E., Ed.; Academic Press: New York, 2013; Vol. 44, pp 101−210.

(15) Thompson, G. D.; Dutton, R.; Sparks, T. C. Spinosad − a case study: an example from a natural products discovery program. *Pest Manage. Sci.* **2000**, *56*, 696−702.

(16) Dripps, J. E.; Boucher, R. E.; Chloridis, A.; Cleveland, C. B.; DeAmicis, C. V.; Gomez, L. E.; Paroonagian, D. L.; Pavan, L. A.; Sparks, T. C.; Watson, G. B. The spinosyn insecticides. In *Green Trends in Insect Control*; Lopez, O., Fernadez-Bolanos, J. G., Eds.; RSC Publishing: Cambridge, UK, 2011; pp 163−212.

(17) Sparks, T. C.; Crouse, G. D.; Dripps, J. E.; Anzeveno, P.; Martynow, J.; Gifford, J. Artificial neural network-based QSAR and the discovery of spinetoram. *J. Comput.-Aided Mol. Des.* **2008**, *22*, 393−401.

(18) Kornis, G. I. Avermectins and milbemycins. In *Agrochemicals from Natural Products*; Godfery, C. R. A., Ed.; Dekker: New York, 1995; pp 215−255.

(19) Fischer, M. H. Structure-activity relationships of the avermectins and milbemycins. In *Phytochemicals for Pest Control*; Hedin, P. A., Hollingworth, R. M., Masler, E. P., Miyamoto, J., Thompson, D. G., Eds.; American Chemical Society: Washington, DC, USA, 1997; pp 220−238.

(20) Mrozik, H.; Linn, B. O.; Eakola, P.; Lusi, A.; Matzuk, A.; Preiser, F. A.; Ostlind, D. A.; Schaeffer, J. M.; Fisher, M. H. Synthesis and biological activities of 13-substituted avermectin aglycones. *J. Med. Chem.* **1989**, *32*, 375−381.

(21) Pitterna, T. Chloride channel activators/new natural products: avermectins and milbemycins. In *Modern Crop Protection Compounds*, 2nd ed.; Kramer, W., Schirmer, U., Jeschke, P., Witschel, M., Eds.; Wiley-VCH: New York, 2012; Vol. 3, pp 1305−1326, DOI: 10.1002/9783527644179.ch32.

(22) Zhao, J.-H.; Xu, X.-J.; Ji, M.-H.; Cheng, J.-L.; Zhu, G.-N. Design, synthesis, and biological activities of milbemycin analogues. *J. Agric. Food Chem.* **2011**, *59*, 4836−4850.

(23) Xu, X.; Lai, S.; Ji, M.; Zhu, G.; Zhao, J. Synthesis, characterization and insecticidal activity of milbemycin analogues. *Youji Huaxue* **2012**, *32*, 1084−1092.

(24) Sparks, T. C.; Dripps, J. E.; Watson, G. B.; Paroonagian, D. Resistance and cross-resistance to the spinosyns − a review and analysis. *Pestic. Biochem. Physiol.* **2012**, *102*, 1−10.

(25) Tietze, L. F.; Schutzenmeister, N.; Grube, A.; Scheffer, T.; Baag, M. M.; Granitzka, M.; Stalke, D. Synthesis of spinosyn analogues for modern crop protection. *Eur. J. Org. Chem.* **2012**, *29*, 5748−5756.

(26) Tietze, L. F.; Sietz, S.; Schutzenmeister, N.; Biller, S.; Hierold, J.; Scheffer, T.; Baag, M. M. Selective glycosylation with the amino sugar D-forosamine for synthesis of spinosyns and its analogues. *Eur. J. Org. Chem.* **2013**, *2013*, 7305−7312.

(27) Anzeveno, P. B.; Green, F. R., III. Rhamnose replacement analogs of spinosyn A. In *Synthesis and Chemistry of Agrochemicals VI*; Baker, D. R., Fenyes, J. G., Lahm, G. P., Selby, T. P., Stevenson, T. M., Eds.; American Chemical Society: Washington, DC, USA, 2002; pp 262−276.

(28) Jeschke, P.; Velten, R.; Ebbinghaus-Kintscher, U.; Beck, M. E.; Losel, P.; Malsam, O.; Eberz, G.; Mohrle, V.; Frode, R. Synthesis of 9-keto-spinosyn derivatives for use in agriculture as pesticides. Bayer CropScience, Germany, Patent, DE 2003-10301519, Jan 17, 2003; 85 pp.

(29) Clark, M.; Cramer, R. D., III; von Opdenbosch, N. Validation of the general purpose Tripos 5.2 force field. *J. Comput. Chem.* **1989**, *10*, 982−1012.

(30) Gasteiger, J.; Marsili, M. Iterative partial equalization of orbital electronegativity: a rapid access to atomic charges. *Tetrahedron* **1980**, *36*, 3219−3222.

(31) Purcel, W. P.; Singer, J. A. A brief review and table of semiempirical parameters used in the Hückel molecular orbital method. *J. Chem. Eng. Data* **1967**, *12*, 235−246.

(32) Finney, D. J. *Probit Analysis*, 3rd ed; Cambridge University Press: Cambridge, UK, 1971.

(33) Sheehan, L. S.; Lill, R. E.; Wilkinson, B.; Sheridan, R. M.; Vousden, W. A.; Kaja, A. L.; Crouse, G. D.; Gifford, J.; Graupner, P.; Karr, L.; Lewer, L.; Sparks, T. C.; Leadlay, P. F.; Waldron, C.; Martin, C. J. Engineering of the spinosyn PKS: directing starter unit incorporation. *J. Nat. Prod.* **2006**, *69*, 1702−1710.

(34) Creemer, L. C.; Kirst, H. A.; Paschal, J. W. Conversion of spinosyn A and spinosyn D to their respective 9- and 17-pseudoaglycones and their aglycones. *J. Antibiot.* **1998**, *51*, 795−800.

(35) Crouse, G. D.; Sparks, T. C.; Schoonover, J.; Gifford, J. M.; Bruce, T.; Worden, T. V.; Martynow, J. G. Recent advances in the chemistry of the spinosyns. *Pest Manage. Sci.* **2001**, *57*, 177−185.

(36) Sparks, T. C.; Crouse, G. D.; Durst, G. Natural products as insecticides: the biology, biochemistry and quantitative structure

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577

activity relationships of spinosyns and spinosoids. *Pest Manage. Sci.* **2001**, *57*, 896−905.
(37) Crouse, G. D.; Sparks, T. C.; DeAmicis, C. V.; Kirst, H. A.; Martynow, J. G.; Creemer, L. L.; Worden, T. V.; Anzeveno, P. B. Chemistry and insecticidal activity of the spinosyns. In *Pesticide Chemistry and Bioscience: The Food-Environment Challenge*; Brook, G. T., Roberts, T. R., Eds.; Royal Society of Chemistry: Cambridge, UK, 1999; pp155−166.
(38) Kirst, H. A.; Creemer, L. C.; Broughton, M. C; Huber, M. L. B.; Turner, J. R. Chemical and microbial modifications of spinosyn: exploring synergies between fermentation microbiology and organic chemistry. In *Synthesis and Chemistry of Agrochemicals VI*; Baker, D. R., Fenyes, J. G., Lahm, G. P., Selby, T. P., Stevenson, T. M., Eds.; American Chemical Society: Washington, DC, USA, 2002; pp 251−261.
(39) Daeuble, J.; Sparks, T. C.; Johnson, P.; Graupner, P. R. Modification of the butenyl-spinosyns utilizing cross-metathesis. *Bioorg. Med. Chem.* **2009**, *17*, 4197−4205.
(40) Chen, X.; Li, L.; Zhang, X.; Zhao, L. Chemical synthesis of spinosad derivatives. Wuhan University of Light Industry, People;s Republic of China, Patent CN 2013-10613847, Nov 26, 2013; 10 pp.
(41) Shi, D.; Feng, X.; Zhung, X.; Chai, H.; Liu, T.; Zhang, Q.; Li, J. Study of synthesis and biological activities of novel macrolides derivatives. *Youji Huaxue* **2014**, *34*, 2543−2550.
(42) Tietze, L. F.; Brasche, G.; Grube, A.; Boknke, N.; Stradler, C. Synthesis of novel spinosyn analogues by Pd-mediated transformations. *Chem. - Eur. J.* **2007**, *13*, 8543−8563.

DOI: 10.1021/acs.jafc.5b01987
J. Agric. Food Chem. 2015, 63, 5571−5577