Conversion of Spinosyn A and Spinosyn D to Their Respective 9- and 17-Pseudoaglycones and Their Aglycones†

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Forosamine at the 17-position of spinosyns A and D was hydrolyzed under mild acidic conditions to give the corresponding 17-pseudoaglycones. The tri-*O*-methylrhamnose at the 9-position of the 17-pseudoaglycone of spinosyn A was hydrolyzed under more vigorous acidic conditions to give the aglycone of spinosyn A. However, these conditions led to decomposition of the 17-pseudoaglycone of spinosyn D, presumably due to more facile protonation of the 5,6-double bond to produce a tertiary carbonium ion which undergoes further rearrangements. Spinosyns J and L (3′-*O*-demethyl spinosyn A and D, respectively) obtained from fermentation of biosynthetically-blocked mutant strains of *Saccharopolyspora spinosa*, were oxidized to give the corresponding 3′-keto-derivatives and the resultant keto-sugars were then β-eliminated under basic conditions to give the 9-pseudoaglycones of spinosyns A and D respectively. Forosamine at the 17-position of the 9-pseudoaglycone of spinosyn D was then readily hydrolyzed to yield the aglycone of spinosyn D.

Spinosyns A (**1**) and D (**2**), formerly known as A83543A and A83543D, are two new commercially important macrolides and the most abundant of the 24 naturally occurring factors produced by the soil microorganism *Saccharopolyspora spinosa*.[1,2] Together, in a mixture of approximately 85% **1** and 15% **2**, they constitute the main active components of the new Dow Agrosciences insecticide *Tracer*[3] and are potent insecticides against such crop pests as tobacco budworm.[4] To initially establish the absolute configuration of **1** and to then provide new starting materials for further derivatization and semi-synthesis of new potentially useful insecticides, the two different sets of pseudoaglycones along with each of the aglycones were sought by selective degradation of the parent spinosyns. Our previous work had described the hydrolysis of the amino sugar forosamine from spinosyn A, giving the 17-pseudoaglycone of spinosyn A (**3**) and D-forosamine; the identity of the latter was confirmed by comparison to a sample of D-forosamine isolated from the natural product spiramycin under similar mild acidic conditions, and permitted the assignment of absolute stereochemistry to spinosyn A.[1] This earlier work has now been extended to the 17-pseudoaglycone of spinosyn D, the production of the 9-pseudoaglycones of both spinosyns A and D, and the formation of the aglycones of both spinosyn A and D.

Results

Hydrolysis of the amino sugar forosamine from either **1** or **2** was conducted using dilute aqueous sulfuric acid at 80°C. This procedure yielded the 17-pseudoaglycones of spinosyn A and D (**3** and **4**) respectively after filtration as a precipitate. D-Forosamine was isolated from the mother liquor by adjusting the pH to 8 with solid sodium bicarbonate, lyophilizing the aqueous solution, and triturating the residue with diethyl ether. Further hydrolysis of **3** using the more vigorous conditions of 7.2 N sulfuric acid in methanol at reflux yielded the

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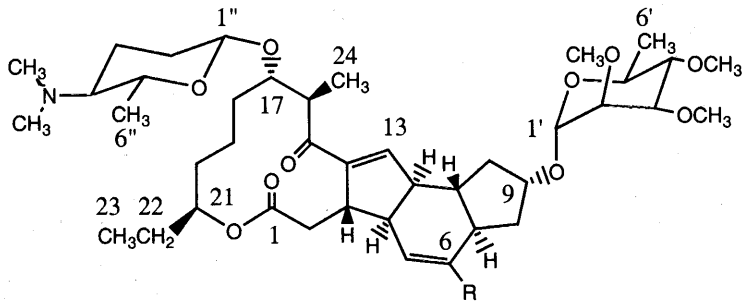
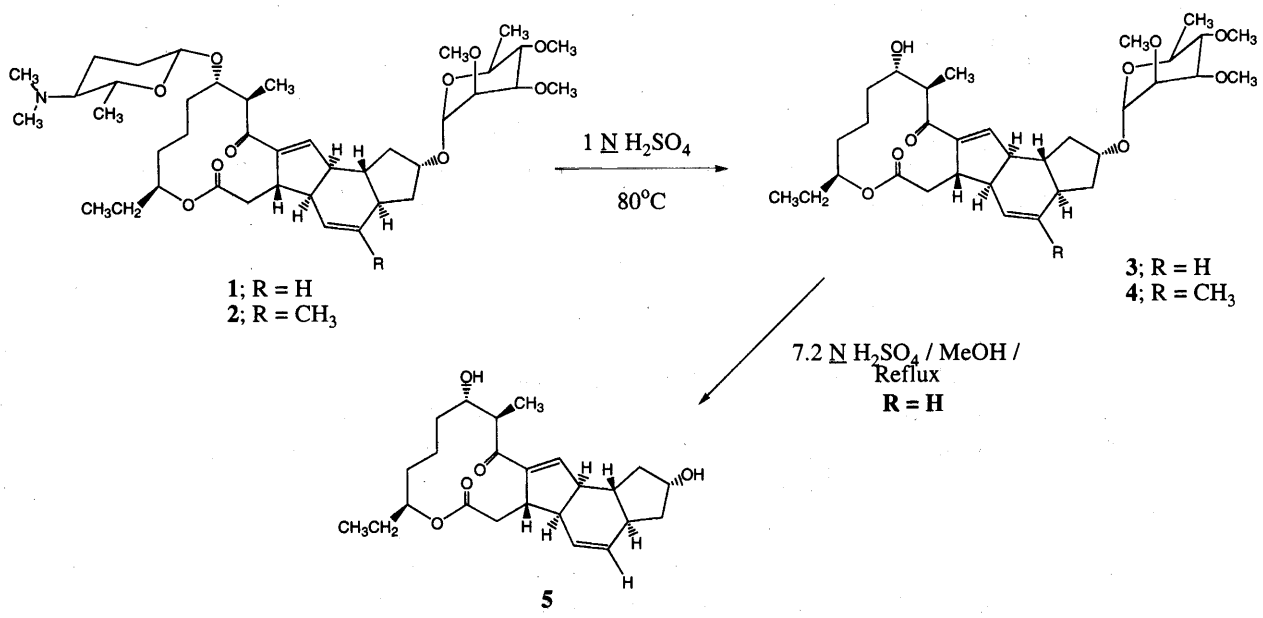
Fig. 1. The structure and numbering of spinosyns A (**1**) and D (**2**).



1: Spinosyn A; R=H
2: Spinosyn D: R=CH_3

Fig. 2. Hydrolysis of **1** and **2** to yield their 17-pseudoaglycones (**3** and **4**) with further hydrolysis of **3** to its aglycone (**5**).



1; R = H
2; R = CH_3

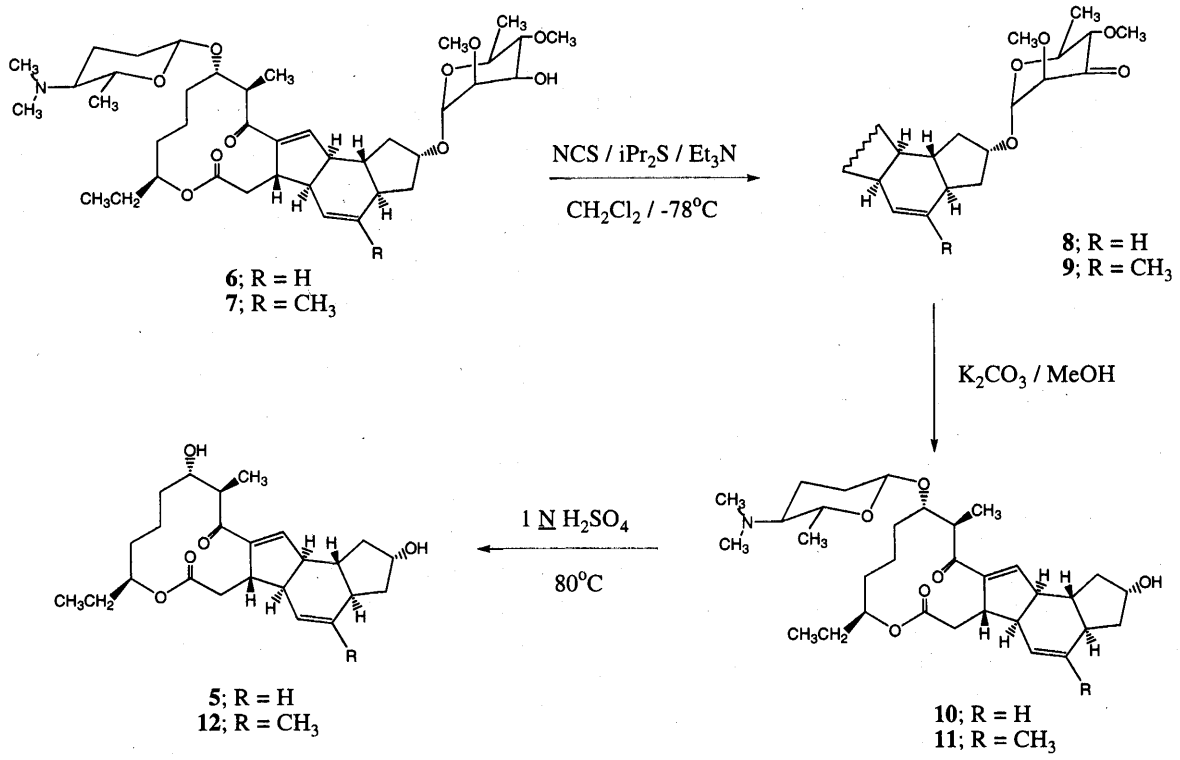
3; R = H
4; R = CH_3

5

aglycone of spinosyn A (**5**) and methyl α-L-(2,3,4-tri-*O*-methyl)rhamnoside.[5] The absolute configuration of the isolated tri-*O*-methylrhamnoside agreed with that predicted from the absolute stereochemistry of **1**; this assignment was further confirmed by its identical optical rotation with that of authentic material produced by the exhaustive methylation of L-rhamnose.[6]

However, when these conditions for acidic hydrolysis were attempted on the 17-pseudoaglycone of spinosyn D to liberate its aglycone, complete decomposition of the molecule occurred. Multiple products were progressively detected in the ^{13}C NMR spectra of the reaction mixture during this decomposition, giving multiple signals attributed to vinylogous carbon atoms. The probable cause for this decomposition is more facile protonation of the 5,6-double bond in **4**, compared to **1**, allowing double-bond migration and subsequent decomposition. Unlike **3**, where the C-6 position is not substituted with a methyl group and decomposition does not occur, the C-6 methyl group of **4** may more readily allow formation of a tertiary carbonium ion at this position. Thus, due to the greater hydrolytic stability of tri-*O*-methylrhamnose relative to forosamine and the decomposition of **4** under strongly acidic conditions, a different approach had to be developed to remove tri-*O*-methylrhamnose under non-acidic conditions while leaving the forosamine moiety intact. Such an approach was achieved by using analogs in which the tri-*O*-

Fig. 3. Oxidation of spinosyns J (**6**) and L (**7**) and the β-elimination of the rhamnose sugar under basic conditions to yield their 9-pseudoaglycones (**10** and **11**), and subsequent hydrolysis of **11** to the aglycone of spinosyn D (**12**).



6; R = H
7; R = CH_3

8; R = H
9; R = CH_3

10; R = H
11; R = CH_3

5; R = H
12; R = CH_3

methylrhamnose had been modified in a way that introduced a chemical handle to this part of the structure.

Extensive strain development efforts on *S. spinosa* had given rise to a mutant strain whose 3′-*O*-methyltransferase had been biosynthetically blocked, thereby allowing production of larger quantities of the original minor factors, spinosyn J (**6**) and spinosyn L (**7**).[7] These two factors were each successfully oxidized under Swern-type conditions using *N*-chlorosuccinimide, diisopropylsulfide and triethylamine in dichloromethane at $-78°C$ to yield their 3′-keto analogs **8** and **9**. These two keto derivatives upon treatment with potassium carbonate in methanol at room temperature then yielded the 9-pseudoaglycones **10** and **11** respectively by β-elimination of the oxidized rhamnose sugar. Since spinosyns A (**1**) and J (**6**) differ only in their 9-*O*-rhamnosyl moiety, their 9-pseudoaglycones are identical. Similarly spinosyns D and L have identical 9-pseudoaglycones. Thus, formation of **10** and **11** produced the desired 9-pseudoaglycones of spinosyn A (J) and spinosyn D (L). Finally the aglycone of spinosyn D (**12**) was then readily formed by the mild hydrolysis of forosamine which, under these acidic conditions, did not cause decomposition.

This chemistry readily allows formation of each of the possible pseudoaglycones and aglycones of the two major family types of spinosyns (spinosyns A and D). In particular, it provides an elegant method for cleaving the more acid-stable sugar from the spinosyns while leaving the less acid-stable sugar (forosamine) intact. Furthermore, it provides a convenient method for producing the strong-acid-labile aglycone of spinosyn D.

Although the parent spinosyns A and D show potent insecticidal activity against tobacco budworm and other major crop pests, all of the pseudoaglycones and aglycones described in this paper were found to have no insecticidal activity at the highest concentrations tested (64 ppm). Consequently, both sugar substituents of the spinosyns are required for their insecticidal activity.

Experimental

General Methods

NMR spectra were determined on a Bruker AMX-500 NMR spectrometer. Mass spectra were obtained on a Finnigan MAT 731 mass spectrometer interfaced to a Finnigan MAT SS-200 data system. Infrared spectra were

Table 1. The ^{1}H NMR assignments for spinosyn A (**1**), the 17-pseudoaglycone of spinosyn A (**3**), the aglycone of spinosyn A (**5**), and the 9-pseudoaglycone of spinosyn A (**10**) in acetone-d_6.[a]

Position	1	3	5	10
2	3.07/2.45	3.07/2.45	3.07/2.44	3.08/2.45
3	2.94	2.94	2.94	2.94
4	3.48	3.50	3.47	3.48
5	5.86	5.87	5.83	5.85
6	5.89	5.91	5.88	5.91
7	2.15	2.15	2.22	2.23
8	1.99/1.34	1.98/1.38	2.31/1.23	2.34/1.25
9	4.31	4.33	4.33	4.34
10	2.36/1.36	2.34/1.35	1.81/1.38	1.78/1.44
11	0.93	0.94	0.89	0.91
12	2.87	2.86	2.81	2.84
13	7.01	7.01	6.97	7.03
16	3.30	3.23	3.22	3.31
17	3.53	3.53	3.53	3.55
18	1.50	1.52/1.43	1.58	1.52
19	1.78/1.17	1.69/1.22	1.69/1.22	1.80/1.19
20	1.51	1.59/1.39	1.47	1.52
21	4.66	4.66	4.64	4.66
22	1.48	1.48	1.45	1.48
23	0.81	0.80	0.80	0.81
24	1.12	1.16	1.14	1.13
1′	4.81	4.83	—	—
2′	3.51	3.54	—	—
3′	3.37	3.39	—	—
4′	3.00	3.02	—	—
5′	3.48	3.50	—	—
6′	1.18	1.18	—	—
2′-OCH_3	3.41	3.43	—	—
3′-OCH_3	3.37	3.40	—	—
4′-OCH_3	3.45	3.47	—	—
1″	4.45	—	—	4.47
2″	1.92/1.37	—	—	1.94/1.39
3″	1.84/1.52	—	—	1.84/1.52
4″	2.12	—	—	2.13
5″	3.56	—	—	3.58
6″	1.21	—	—	1.21
$N(CH_3)_2$	2.22	—	—	2.22

[a] The analogous spinosyn D compounds are identical except for the expected differences in the 5 and 6 positions due to the presence of a methyl group at the 6-position.

determined on a Nicolet 510P spectrometer. UV spectra were measured on a Shimadzu UV-2101 PC Spectrophotometer. The preparation of spinosyns A, D, J, and L were carried out in the Lilly fermentation facilities at Indianapolis, IN., USA. All other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI., USA.

Synthesis of the 17-Pseudoaglycone of Spinosyn A (3)

Spinosyn A (20.0 g, 27.4 mmol) was dissolved in 300 ml of 1 N H_2SO_4 and heated to 80°C with mechanical stirring for 2 hours. The reaction was then cooled to room temperature. The precipitate was filtered with suction and washed with fresh 1 N H_2SO_4. The solid product was then dissolved in dichloromethane, washed with brine, dried over K_2CO_3, and evaporated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with 70% EtOAc in hexane, to give **3** (14.5 g; 89%) as a colorless glass; FD-MS *m/z* 1182 ($2M^+$), 593 ($M+2H^+$), 591 (M^+); UV λ_{max} nm (ε) 243 (9,440); IR ($CHCl_3$) cm^{-1} 3460, 2968, 2932, 2879, 1721, 1660, 1372, 1215, 1162, 1152, 1140, 1119, 1104, 1056, 1036; ^{1}H NMR ($CDCl_3$) see Table 1.

Synthesis of the 17-Pseudoaglycone of Spinosyn D (4)

The reaction was run as with **3** starting with spinosyn D (1.0 g, 1.34 mmol) and giving **4** (547 mg; 68%) as a colorless glass; FD-MS *m/z* 604 (M^+); UV λ_{max} nm (ε) 239 (9,079); IR ($CHCl_3$) cm^{-1} 3500, 3024, 2936, 1715, 1659, 1374, 1117, 1039; ^{1}H NMR ($CDCl_3$) see Table 1.

Synthesis of Spinosyn A Aglycone (5)

To a solution of **3** (6.03 g, 10.7 mmol) in MeOH (267 ml), 7.2 N H_2SO_4 (396 ml) was added and the solution was heated to reflux for 3 hours. The mixture was then cooled in an ice bath. A large amount of $NaHCO_3$ (solid) and saturated aqueous $NaHCO_3$ were added cautiously; however, the pH was never brought above 1.0. The aqueous solution was mixed with Et_2O and separated. The aqueous portion was then extracted with fresh Et_2O. The Et_2O extracts were combined, washed with brine, dried with K_2CO_3 and evaporated at reduced pressure. The resulting yellow semi-solid (4.89 g) was purified by silica gel chromatography, eluting with 100% dichloromethane and then a gradient up to 7.5% MeOH in dichloromethane, giving **5** (2.83 g, 66% yield) as a slightly unstable colorless glass; FD-MS *m/z* 403 (M^+), 402 ($M\text{-}H^+$); UV λ_{max} nm (ε) 243 (7680); IR ($CHCl_3$) cm^{-1} 3440, 2965, 2932, 1718, 1658, 1372, 1227, 1215, 1164; ^{1}H NMR ($CDCl_3$) see Table 1.

Synthesis of 3′-Des-*O*-methyl-3′-keto Spinosyn A (8)

To a suspension of *N*-chlorosuccinimide (104.7 mg, 0.78 mmol) in dichloromethane (2.6 ml) cooled to −78°C under nitrogen, diisopropyl sulfide (125 μl, 0.86 mmol) was added, and the mixture was stirred at −78°C. After 0.5 hours, spinosyn J (184.6 mg, 0.26 mmol) dissolved in dichloromethane (1 ml) was added slowly. When the

addition was complete, the solution was stirred at −78°C for 6.25 hours. Triethylamine (109 μl, 0.78 mmol) was then added, and the solution was warmed to room temperature, giving a red color. After warming, Et_2O (6 ml) was added and a precipitate formed. Dichloromethane was added to dissolve the precipitate and was then combined with the Et_2O solution, washed with 0.1 N HCl, and then brine. The organic layer was then dried with $MgSO_4$ and evaporated at room temperature. The resulting colorless glass (215 mg) was semi-purified by flash chromatography, eluting with 5% MeOH in dichloromethane, giving **8** as a colorless semi-solid (151.2 mg). This product was contaminated with diisopropyl sulfide, but the product was used without further purification; FD-MS *m/z* 716 ($M+H^+$); IR ($CHCl_3$) cm^{-1} 2973, 2937, 1715, 1650, 1460, 1380, 1234, 1142, 1110, 1061, 1011.

Synthesis of 3'-Des-*O*-methyl-3'-keto Spinosyn D (**9**)

The reaction was run as with **8**, starting with spinosyn L (997.4 mg, 1.36 mmol), giving **9** (850 mg) as a colorless semi-solid. This product was contaminated with diisopropyl sulfide, but the product was used without further purification; FD-MS *m/z* 729 (M^+); IR ($CHCl_3$) cm^{-1} 3018, 2974, 2937, 1715, 1658, 1457, 1233.

Synthesis of the 9-Pseudoaglycone of Spinosyn A (**10**)

To a solution of **8** (1.89 g, 2.64 mmol) in MeOH (100 ml), K_2CO_3 (anhydrous; 1.82 g, 13.2 mmol) was added and the mixture was stirred at room temperature for 1 hour. Et_2O (100 ml) was then added and the mixture was filtered. The filtrate was evaporated at room temperature giving a yellow solid. The yellow solid was dissolved in dichloromethane and washed with water, then brine, and dried with $MgSO_4$. The dichloromethane was then evaporated at reduced pressure, giving a colorless semi-solid (1.53 g). This semi-solid was purified by flash chromatography using a one-step gradient of 5% MeOH in dichloromethane to 10% MeOH in dichloromethane, giving **10** (1.09 g, 76% yield) as an off-white glass; FD-MS *m/z* 544 (M^+); UV λ_{max} nm (ε) 244 (9,754); IR ($CHCl_3$) cm^{-1} 3617, 3017, 2974, 1714, 1658, 1606, 1456, 1373, 1233, 1164, 1123, 1063, 989, 877; ^1H NMR ($CDCl_3$) see Table 1.

Synthesis of the 9-Pseudoaglycone of Spinosyn D (**11**)

The reaction was run as with **10**, starting with **9** (770 mg, 1.06 mmol) and giving **11** (246.4 mg; 42%) as a colorless glass; FD-MS *m/z* 557 ($M-H^+$), 558 (M^+), 559 ($M+H^+$); UV λ_{max} nm (ε) 245 (10,280), 201 (10,690); IR ($CHCl_3$) cm^{-1} 3455, 2968, 2939, 2878, 2828, 2782, 1723, 1660, 1610, 1457, 1373, 1258, 1163, 1125, 1070, 989, 902; ^1H NMR ($CDCl_3$) see Table 1.

Synthesis of Spinosyn D Aglycone (**12**)

To a suspension of **11** (132 mg, 0.24 mmol) in water (5 ml), 1 N H_2SO_4 was added dropwise to a pH of 1.7 whereupon the mixture became homogeneous. This solution was heated to 80°C for 3.75 hours, during which time an oil separated from the solution. The mixture was cooled to room temperature and dichloromethane was added to dissolve the oil. The aqueous was separated and extracted with fresh dichloromethane. The dichloromethane was combined, washed quickly with 1 N H_2SO_4, dried with K_2CO_3 and evaporated at room temperature giving a pale yellow glass (82.9 mg). The product was purified by flash chromatography, eluting with 5% MeOH in dichloromethane, giving **12** (63.6 mg, 63% yield) as a slightly unstable colorless glass; FD-MS *m/z* 416 (M^+), 417 ($M+H^+$); UV λ_{max} nm (ε) 244 (9,932); IR ($CHCl_3$) cm^{-1} 3466, 2994, 2877, 1722, 1653, 1643, 1457, 1438, 1376, 1164, 1077, 1010, 907; ^1H NMR ($CDCl_3$) see Table 1.

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