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NATURAL-PRODUCT-BASED INSECTICIDAL AGENTS 16. SEMISYNTHESIS OF C7-OXIME SULFONATE ESTER DERIVATIVES OF OBACUNONE AS INSECTICIDAL AGENTS AGAINST *MYTHIMNA SEPARATA* WALKER

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Dedicated to Professor Dr. Isao Kuwajima on the occasion of his 77th birthday

Abstract – A series of novel C7-oxime sulfonate ester derivatives of obacunone (**3a-f**) were synthesized. Their insecticidal activity was also evaluated at the concentration of 1 mg/mL against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest. Especially C7-oxime *p*-ethylphenylsulfonate ester of obacunone (**3d**) exhibited more potent insecticidal activity than their precursor obacunone and toosendanin (a positive control).

Without agrochemicals, 30% of the world's crops would be lost before they were harvested,[1] therefore, the routine use of a wide variety of synthetic insecticides in agriculture is still the most effective method for controlling insect pests. However, the repeat application of those agrochemicals over the years has resulted in the development of resistance in insect pest populations and environmental problems.[2,3] Recently, for botanical insecticides are originated from plant secondary metabolites from the interaction between plants and environment (life and non-life) during the long period of evolution in plants, research and development of botanical insecticides have received more and more attention.[4-6]

Obacunone (**1**, Figure 1) was isolated from many species of plants such as *Citrus* and *Dictamnus angustifolius*.[7] Compound **1** showed many medicinal activities such as antiproliferative,[8] anticancer,[9-11] antimalarial,[12] and antioxidant activities.[13] On the other hand, compound **1** also displayed interesting

insecticidal activity.[14,15] Recently, we have prepared a series of 4′-substituted benzenesulfonate derivatives of 4-deoxypodophyllotoxin as insecticidal agents, and found some compounds exhibited the more potent insecticidal activity than toosendanin, a commercial botanical insecticide from *Melia azedarach*.[16] Encouraged by the above results, and in continuation of our program aimed at the discovery and development of new potent natural-product-based insecticidal agents,[16-19] herein we synthesized a series of C7-oxime sulfonate ester derivatives of obacunone (**3a-f**, Scheme 1). Subsequently, their insecticidal activity was evaluated against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker), an important and typical lepidopteran pest.

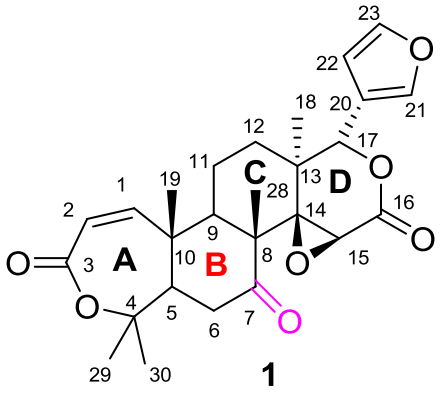
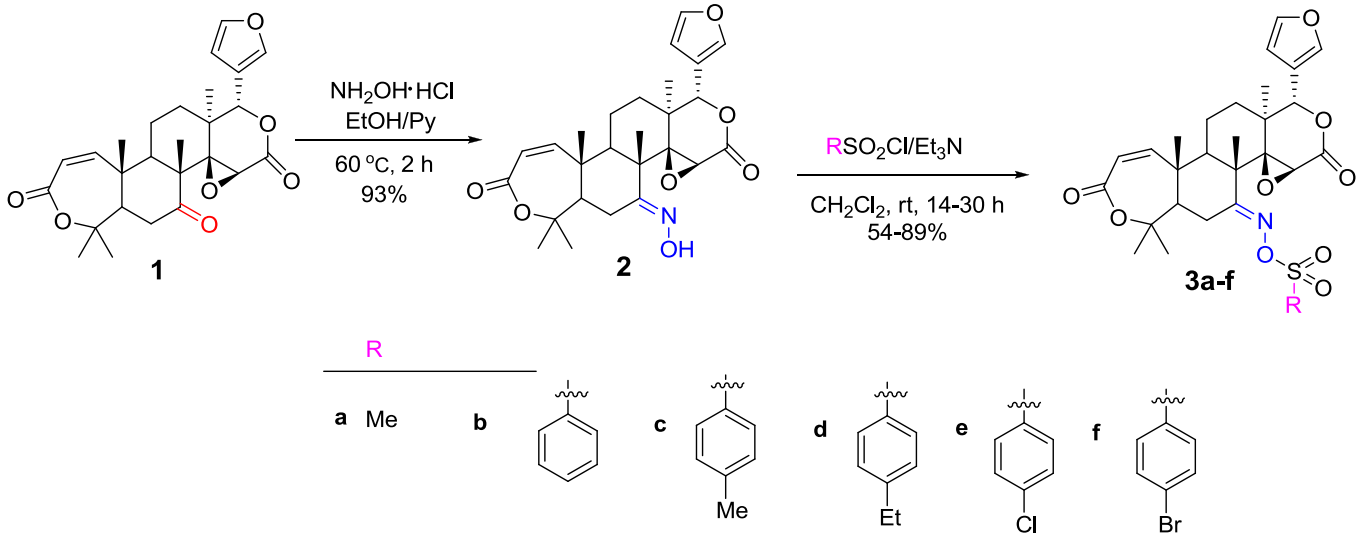


Figure 1. Chemical structure of obacunone (**1**)



Scheme 1. Synthesis of C7-oxime sulfonate ester derivatives of obacunone (**3a-f**)

As shown in Scheme 1, first, obacunone (**1**) reacted with hydroxylamine hydrochloride to afford C7-oximeobacunone (**2**) in a 93% yield. Then **2** reacted with different sulfonyl chlorides in the presence of triethylamine to smoothly give C7-oxime sulfonylester derivatives of obacunone (**3a-f**) in 54-89% yields. The structures of all target compounds were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp.

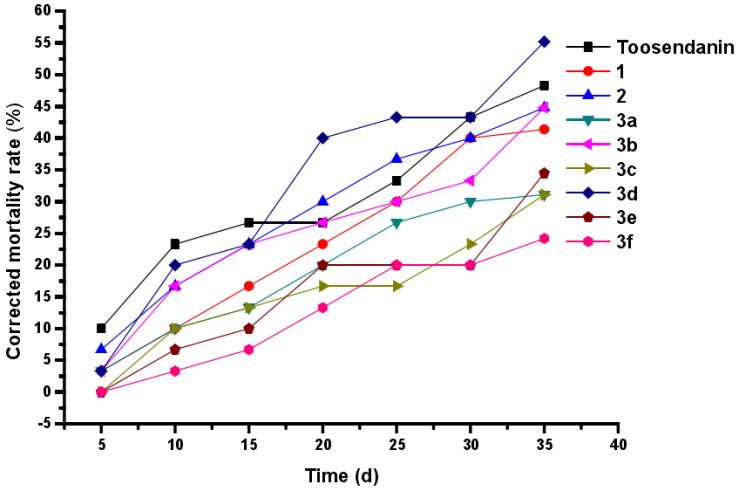


Figure 2. Corrected mortality rates of *M. separata* caused by **3a-f** with the increase of time

Table 1. Insecticidal activity of **3a-f** against *M. separata* on leaves treated with a concentration of 1 mg/mL

Compounds	Corrected mortality rate (%)		
	10 d	20 d	35 d
1	10.0 ± 0	23.3 ± 3.3	41.4 ± 3.3
2	16.7 ± 3.3	30.0 ± 0	44.8 ± 3.3
3a	10.0 ± 5.8	20.0 ± 0	31.1 ± 6.7
3b	16.7 ± 3.3	26.7 ± 6.7	44.8 ± 3.3
3c	10.0 ± 0	16.7 ± 3.3	31.1 ± 6.7
3d	20.0 ± 5.8	40.0 ± 5.8	55.2 ± 3.3
3e	6.7 ± 6.7	20.0 ± 0	34.5 ± 6.7
3f	3.3 ± 3.3	13.3 ± 3.3	24.2 ± 3.3
toosendanin	23.3 ± 3.3	26.7 ± 3.3	48.3 ± 5.8
blank control	0 ± 0	0 ± 0	3.3 ± 3.3

The insecticidal activity of compounds **1**, **2** and **3a-f** against the pre-third-instar larvae of *Mythimna separata* was tested at the concentration of 1 mg/mL by leaf-dipping method.[16] Toosendanin, a commercial insecticide derived from *Melia azedarach*, was used as a positive control at 1 mg/mL. The corrected mortality rates of *M. separata* caused by **1**, **2** and **3a-f** with the advance of time were outlined in Figure 2. The corresponding mortality rates after 35 days were higher than those after 10 and 20 days. Therefore, these compounds, in a time-dependent manner, different from other conventional neurotoxic

insecticides such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity. For example, the corrected mortality rate of **3d** against *M. separata* after 10 days was 20%, after 20 days the corresponding mortality rate was increased to 40%, but after 35 days the corresponding mortality rate was increased to 55.2% (Table 1). Meanwhile, the symptoms of the tested *M. separata* were also characterized in the same way as our previous reports.[16-18] The pupation of the larvae and the adult emergence of *M. separata* were inhibited by these compounds, therefore, the stage from the larvae to adulthood of *M. separata* was prolonged as compared with the control group. Moreover, for feeding too much leaves treated with these tested compounds during the first 48 h, some larvae died slowly with the slim and wrinkled bodies; many larvae of the treated groups also moulted to malformed pupae, and died during the stage of pupation; malformed moths with imperfect wings were observed in the treated groups.

As shown in Table 1, C7-oxime *p*-ethylphenylsulfonate ester of obacunone (**3d**) exhibited more potent insecticidal activity than their precursors (**1** and **2**) and toosendanin (a positive control). Introduction of the ethyl group at the C-4 position on the phenyl ring of **3b** could afford the potent compound **3d**, the final mortality rate of which was 55.2%; whereas introduction of the methyl (electron-donating group) or chlorine/bromine (electron-withdrawing groups) on the phenyl ring of **3b** led to the less active compounds **3c**, **3e** and **3f** (e.g., the final mortality rates: 31.1% for **3c**; 34.5% for **3e**; 24.2% for **3f**).

In conclusion, we have prepared a series of novel C7-oxime sulfonate ester derivatives of obacunone. Their insecticidal activity was tested against the pre-third-instar larvae of *Mythimna separata in vivo*. Among all derivatives, C7-oxime *p*-ethylphenylsulfonate ester of obacunone (**3d**) exhibited more potent insecticidal activity than their precursor obacunone and toosendanin (a positive control). The afore-mentioned results will encourage us to further investigate new obacunone derivatives as insecticidal agents.

EXPERIMENTAL

All reagents and solvents were of reagent grade or purified according to standard methods before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China). Silica gel column chromatography was performed with silica gel 200-300 mesh (Qingdao Haiyang Chemical Co., Ltd., China). Melting points (mp) were determined on a XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. Infrared spectra (IR) were recorded on a Bruker TENSOR 27 spectrometer. Optical rotation was measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ on a Bruker Avance 500 MHz instrument, and tetramethylsilane (TMS) was used as the internal standard. High-resolution mass spectra (HR-MS) were obtained on an IonSpec

4.7 Tesla FTMS instrument.

Synthesis of C7-oximeobacunone (2)

A mixture of **1** (0.5 mmol) and hydroxylamine hydrochloride (5.0 mmol) in pyridine (0.5 mL) and absolute EtOH (10 mL) was stirred at 60 ^{o}C for 2 h. When the reaction was complete, checked by TLC analysis, the solvent was removed under reduced pressure and saturated aqueous $NaHCO_3$ (15 mL) was added to the residue, which was extracted with EtOAc (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography eluting with petroleum ether/EtOAc (2:1, v/v) to give **2**. CAS no.123885-85-8; Yield 93%, white solid, mp 268-270 ^{o}C (lit.[20] 268 ^{o}C); $[\alpha]^{20}_{D}$ -12 (*c* 3.3, acetone); IR cm^{-1} (KBr): 3432, 2953, 1752, 1681, 1389, 1267, 1076; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.41 (s, 1H, H-21), 7.39 (s, 1H, H-23), 7.34 (s, 1H, -OH), 6.49 (d, *J* = 11.5 Hz, 1H, H-1), 6.36 (s, 1H, H-22), 5.90 (d, *J* = 11.5 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.67 (s, 1H, H-15), 3.34 (dd, *J* = 14.5, 5.0 Hz, 1H, H-6), 2.30 (dd, *J* = 14.0, 5.0 Hz, 1H, H-5), 2.06-2.15 (m, 2H, H-6, 9), 1.81-1.94 (m, 3H, H-11, 12), 1.53 (s, 3H, H-29), 1.51 (s, 3H, H-28), 1.44-1.48 (m, 1H, H-11), 1.40 (s, 3H, H-19), 1.22 (s, 3H, H-18), 1.13 (s, 3H, H-30); HRMS *m/z* calcd for $C_{26}H_{32}NO_7$ ($[M+H]^+$) 470.2173, found 470.2176.

General procedure for synthesis of C7-oxime sulfonate ester derivatives of obacunone (3a-f).

To a stirred solution of **2** (0.21 mmol) and triethylamine (0.31 mmol) in dry CH_2Cl_2 (10 mL) at room temperature, sulfonyl chloride RSO_2Cl (0.25 mmol) was added. When the reaction was complete according to TLC analysis, water (15 mL) was added to the mixture, which was extracted with CH_2Cl_2 (30 mL). The organic phase was washed by aqueous HCl (0.1 mol/L, 30 mL), 5% aqueous Na_2CO_3 (30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by PTLC to give the pure target products **3a-f** in 54-89% yields.

3a: Yield 81%, white solid, mp 160-162 ^{o}C; $[\alpha]^{20}_{D}$ -4 (*c* 3.0, acetone); IR cm^{-1} (KBr): 2956, 1744, 1704, 1644, 1393, 1285, 1030; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 (s, 1H, H-21), 7.41 (t, *J* = 1.5 Hz, 1H, H-23), 6.47 (d, *J* = 11.5 Hz, 1H, H-1), 6.39 (d, *J* = 1.5 Hz, 1H, H-22), 5.94 (d, *J* = 11.5 Hz, 1H, H-2), 5.55 (s, 1H, H-17), 3.68 (s, 1H, H-15), 3.19-3.22 (m, 4H, H-6, -CH_3), 2.34-2.43 (m, 2H, H-5, 6), 2.16-2.19 (m, 1H, H-9), 1.87-1.97 (m, 3H, H-11, 12), 1.52 (s, 3H, H-28), 1.47-1.51 (m, 4H, H-11, 29), 1.42(s, 3H, H-19), 1.29(s, 3H, H-18), 1.23 (s, 3H, H-30); HRMS *m/z* calcd for $C_{27}H_{34}NO_9S$ ($[M+H]^+$) 548.1949, found 548.1948.

3b: Yield 57%, white solid, mp 178-179 ^{o}C; $[\alpha]^{20}_{D}$ 10 (*c* 4.2, acetone); IR cm^{-1} (KBr): 3032, 2952, 1747, 1702, 1394, 1284, 1074; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.70-7.72(m, 1H, Ar-H), 7.62-7.69 (m, 2H, Ar-H), 7.38-7.40 (m, 2H, H-21, 23), 6.43 (d, *J* = 11.5 Hz, 1H, H-1), 6.31 (d, *J* = 1.0 Hz, 1H, H-22), 5.92 (d, *J* = 12.0 Hz, 1H, H-2), 5.38 (s, 1H, H-17), 3.46 (s, 1H, H-15), 3.22 (dd, *J* = 13.5, 4.0 Hz, 1H, H-6), 2.26-2.38(m, 2H, H-5, 6), 1.99-2.02 (m, 1H, H-9), 1.80-1.87 (m, 3H, H-11, 12),

1.53 (s, 3H, H-28), 1.51 (s, 3H, H-29), 1.38-1.42 (m, 4H, H-11, 19), 1.13 (s, 3H, H-18), 0.86 (s, 3H, H-30); HRMS *m/z* calcd for $C_{32}H_{36}NO_9S$ ($[M+H]^+$) 610.2105, found 610.2120.

3c: Yield 54%, white solid, mp 172-174 °C; $[\alpha]^{20}_D$ 6 (*c* 3.3, acetone); IR cm^{-1} (KBr): 3039, 2953, 1744, 1698, 1632, 1394, 1282, 1075; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.86 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.38-7.43 (m, 4H, H-21, 23, Ar-H), 6.43 (d, *J* = 11.5 Hz, 1H, H-1), 6.32 (s, 1H, H-22), 5.91 (d, *J* = 11.5 Hz, 1H, H-2), 5.38 (s, 1H, H-17), 3.47 (s, 1H, H-15), 3.21 (dd, *J* = 14.0, 4.0 Hz, 1H, H-6), 2.47 (s, 3H, $-CH_3$), 2.24-2.37 (m, 2H, H-5, 6), 2.00-2.04 (m, 1H, H-9), 1.80-1.88 (m, 3H, H-11, 12), 1.53 (s, 3H, H-28), 1.51 (s, 3H, H-29), 1.40-1.46 (m, 1H, H-11), 1.38 (s, 3H, H-19), 1.12 (s, 3H, H-18), 0.98 (s, 3H, H-30); HRMS *m/z* calcd for $C_{33}H_{38}NO_9S$ ($[M+H]^+$) 624.2262, found 624.2251.

3d: Yield 89%, white solid, mp 130-132 °C; $[\alpha]^{20}_D$ 5 (*c* 3.0, acetone); IR cm^{-1} (KBr): 3039, 2966, 1747, 1704, 1634, 1373, 1282, 1123; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.43 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.38-7.40 (m, 2H, H-21, 23), 6.43 (d, *J* = 11.5 Hz, 1H, H-1), 6.31 (d, 1H, *J* = 1.0 Hz, H-22), 5.91 (d, *J* = 11.5 Hz, 1H, H-2), 5.39 (s, 1H, H-17), 3.47 (s, 1H, H-15), 3.22 (dd, *J* = 14.0, 4.0 Hz, 1H, H-6), 2.74 (q, *J* = 7.5 Hz, 2H, $-C\mathit{H}_2CH_3$), 2.25-2.37 (m, 2H, H-5, 6), 1.99-2.01 (m, 1H, H-9), 1.79-1.89 (m, 3H, H-11, 12), 1.53 (s, 3H, H-28), 1.51 (s, 3H, H-29), 1.35-1.43 (m, 4H, H-11, 19), 1.26 (d, *J* = 7.5 Hz, 3H, $-CH_2C\mathit{H}_3$), 1.13 (s, 3H, H-18), 0.88 (s, 3H, H-30); HRMS *m/z* calcd for $C_{34}H_{40}NO_9S$ ($[M+H]^+$) 638.1147, found 638.2349.

3e: Yield 54%, white solid, mp 171-172 °C; $[\alpha]^{20}_D$ 10 (*c* 3.2, acetone); IR cm^{-1} (KBr): 3040, 2954, 1748, 1696, 1633, 1391, 1283, 1086; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.61 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.40-7.41 (m, 2H, H-21, 23), 6.43 (d, *J* = 11.5 Hz, 1H, H-1), 6.34 (s, 1H, H-22), 5.92 (d, *J* = 11.5 Hz, 1H, H-2), 5.39 (s, 1H, H-17), 3.47 (s, 1H, H-15), 3.21 (dd, *J* = 13.5, 3.5 Hz, 1H, H-6), 2.27-2.38 (m, 2H, H-5, 6), 1.99 -2.01 (m, 1H, H-9), 1.79-1.88 (m, 3H, H-11, 12), 1.53 (s, 3H, H-28), 1.51 (s, 3H, H-29), 1.38-1.42 (m, 4H, H-11, 19), 1.14 (s, 3H, H-18), 0.86 (s, 3H, H-30); HRMS *m/z* calcd for $C_{32}H_{35}NO_9SCl$ ($[M+H]^+$) 644.1716, found 644.1699.

3f: Yield 56%, white solid, mp 162-164 °C; $[\alpha]^{20}_D$ 17 (*c* 2.7, acetone); IR cm^{-1} (KBr): 3039, 2951, 1742, 1707, 1654, 1392, 1282, 1071; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.78 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.41-7.42 (m, 2H, H-21, 23), 6.42 (d, *J* = 11.5 Hz, 1H, H-1), 6.35 (s, 1H, H-22), 5.92 (d, *J* = 11.5 Hz, 1H, H-2), 5.39 (s, 1H, H-17), 3.48 (s, 1H, H-15), 3.21 (dd, *J* = 13.5, 3.5 Hz, 1H, H-6), 2.27-2.37 (m, 2H, H-5, 6), 1.98 -2.01 (m, 1H, H-9), 1.80-1.88 (m, 3H, H-11, 12), 1.53 (s, 3H, H-28), 1.51 (s, 3H, H-29), 1.38-1.42 (m, 4H, H-11, 19), 1.14 (s, 3H, H-18), 0.85 (s, 3H, H-30); HRMS *m/z* calcd for $C_{33}H_{31}N_5O_5SBr$ ($[M+H]^+$) 688.1224, found 688.1224.

Bioassay

The insecticidal activity of **1**, **2** and **3a-f** against the pre-third-instar larvae of *M. separata* was assessed by leaf-dipping method as described previously.[17] For each compound, 30 larvae (10 larvae per group) were

used. Acetone solutions of **1**, **2**, **3a-f** and toosendanin (used as a positive control) were prepared at the concentration of 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out and dried in a room. Leaves treated with acetone alone were used as a control group. Several treated leaves were kept in each dish, where every 10 larvae were raised. If the treated leaves were consumed, the corresponding ones were added to the dish. After 48 h, untreated fresh leaves were added to the all dish until the adult emergence. The experiment was carried out at 25 ± 2 ^{o}C and relative humidity (RH) 65-80%, and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *M. separata* was calculated by the following formula:

Corrected mortality rate (%) = $(T-C) \times 100/(100\%-C)$

Where T is the mortality rate in the group treated with the tested compounds, and C is the mortality rate in the blank control group (T and C were all expressed as the percentage).

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