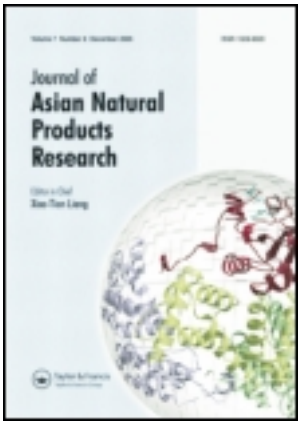


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Synthesis of a novel phosphate analog of 20-hydroxylecdysone with potent hypoglycemic activity

Dan Zhang [a], Min Zhang [a], Bao Ding [b], Xiao-Lin Wang [b], Zong-Yin Qiu [b] & Yong Qin [a]

[a] Department of Medicinal Natural Products and Key Laboratory of Drug Targeting & Drug Delivery Systems of the Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu, 610041, China

[b] Chongqing Zhien Pharmaceutical Co., Ltd, Chongqing, 400039, China

Available online: 30 Mar 2011

To cite this article: Dan Zhang, Min Zhang, Bao Ding, Xiao-Lin Wang, Zong-Yin Qiu & Yong Qin (2011): Synthesis of a novel phosphate analog of 20-hydroxylecdysone with potent hypoglycemic activity, Journal of Asian Natural Products Research, 13:04, 297-303

To link to this article: http://dx.doi.org/10.1080/10286020.2011.554830

Journal of Asian Natural Products Research
Vol. 13, No. 4, April 2011, 297–303

Synthesis of a novel phosphate analog of 20-hydroxylecdysone with potent hypoglycemic activity

Dan Zhang[a], Min Zhang[a], Bao Ding[b], Xiao-Lin Wang[b†]*, Zong-Yin Qiu[b] and Yong Qin[a†]*

[a]*Department of Medicinal Natural Products and Key Laboratory of Drug Targeting & Drug Delivery Systems of the Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu 610041, China;* [b]*Chongqing Zhien Pharmaceutical Co., Ltd, Chongqing 400039, China*

(*Received 4 December 2010; final version received 11 January 2011*)

A novel, water-soluble 20-hydroxylecdysono-20,22-phosphoric acid **2** and its sodium salt **3** were designed and synthesized from 20-hydroxylecdysone **1** in six steps and with 67% overall yield. The synthesized phosphoric acid **2** exhibited hypoglycemic activity >40-fold more potent than that of 20-hydroxylecdysone **1** at concentrations between 2×10^{-7} and 2×10^{-8} mol/l in a glucose consumption test in HepG2 cells. At a concentration of 2×10^{-9} mol/l, phosphoric acid **2** was still active, causing a maximum increase in glucose consumption of more than 500%, while 20-hydroxylecdysone **1** was inactive.

Keywords: 20-hydroxylecdysone; 20-hydroxylecdysone phosphate; hypoglycemic activity

1. Introduction

Ecdysteroids are important natural polyhydroxylated steroid hormones widespread in invertebrates, plants and fungi, and are known to be responsible for molting and metamorphosis in insects [1]. 20-Hydroxylecdysone **1** (Figure 1) [2], the most abundant and representative member of ecdysteroids, was found to show many physiological activities, including hypocholesterolemic [3] and antiarrhythmic activity [4]. In 1971, Yoshida *et al.* [5] first reported the hypoglycemic activity of 20-hydroxylecdysone **1** in rats. Several other groups confirmed the hypoglycemic activity of **1** *in vitro* and *in vivo* [6]. Several patents appeared describing the preparation of antidiabetic agents from extracts of plants containing **1** [7]. These studies suggest that naturally abundant **1** is a good lead for the further development of a hypoglycemic agent.

One disadvantage of **1** as a hypoglycemic reagent is its poor solubility in water. It is well known that hydroxyl phosphorylation is an efficient way to improve the water solubility and biological activity of a lead compound, as long as this modification does not change the key pharmacophores of the lead. Early attempts [8] to chemically modify ecdysone and **1** suggested that the *cis*-fused A/B ring junction, 7-en-6-one functional group, and 14α-hydroxyl group were crucial to biological activity. At the same time, other work showed that a free 22-hydroxyl group in **1** was not essential for high molting activity; indeed,

*Corresponding authors. Emails: sabrina@tom.com; yongqin@scu.edu.cn
†Correspondence should be addressed to X.-L. Wang for bioassay and Y. Qin for synthesis.

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.554830
http://www.informaworld.com

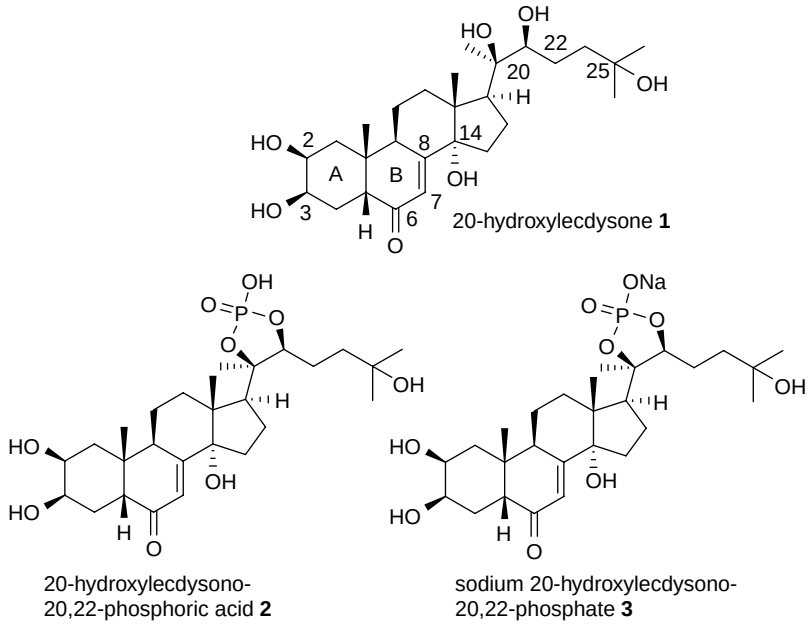


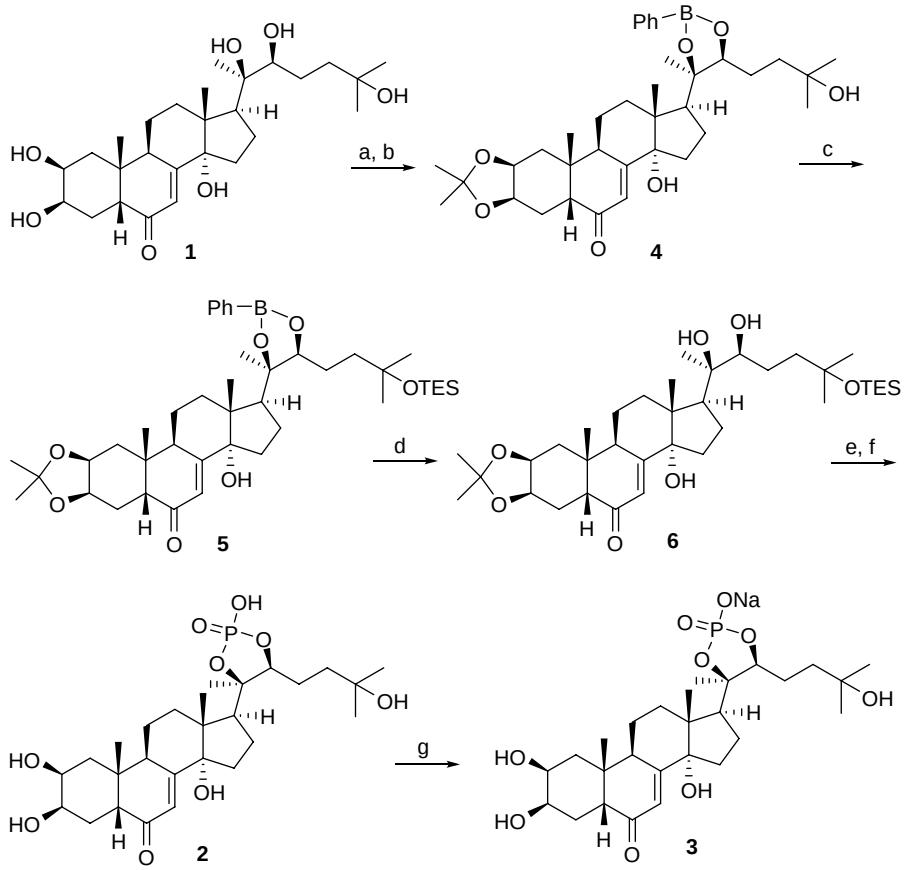
Figure 1. Structures of 20-hydroxylecdysone (**1**), 20-hydroxylecdysono-20,22-phosphoric acid (**2**), and sodium 20-hydroxylecdysono-20,22-phosphate (**3**).

22-*O*-substituted analogs exhibited enhanced activities [9].

2. Results and discussion

Based on these preliminary results, we attempted to enhance the water solubility and hypoglycemic activity of **1** by converting it to a novel 20-hydroxylecdysono-20,22-phosphoric acid (**2**, Figure 1) [10]. Preliminary studies of the hypoglycemic activity of **2** are also reported in this work. The synthetic pathway for **2** is shown in Scheme 1, the key step of which involves selective protection and deprotection of hydroxyl groups at positions 2, 3, 20, 22, and 25. Based on a published method [11], the 20,22-hydroxyl groups of the commercially available **1** were selectively protected with phenyl borate, and the 2,3-hydroxyl groups of the resulting boronic ester were further protected as isopropyl acetonide **4** by reaction with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid, giving 91% yield in two steps. Treatment of **4** with triethylsilyl chloride, imidazole, and catalytic 4-dimethylaminopyridine (DMAP) in dichloromethane at room temperature successfully led to the silylation of the secondary hydroxyl group at position 25 to give **5** in high yield. Oxidative cleavage of the boronic ester in **5** using sodium hydroxide and hydrogen peroxide afforded diol **6** in 98% yield. Phosphorylation of **6** with phosphorus oxychloride and pyridine in dry THF, followed by acidic hydrolysis to remove simultaneously both the triethylsilyl and isopropanyl protecting groups, afforded 20-hydroxylecdysono-20,22-phosphoric acid **2** in 83% yield. Phosphoric acid **2** was readily converted to the corresponding sodium salt **3** by treating **2** with sodium bicarbonate. The synthetic route devised here was highly efficient (67% overall yield), and we were able to scale it up to kilogram quantities.

As we anticipated, both phosphoric acid **2** and its sodium salt **3** were stable, water-soluble white powders. Their solubilities in water, respectively, were 20-fold and 50-fold greater than that of 20-hydroxylecdysone **1**.



Scheme 1. Synthesis of **2** and **3**. Reagents and conditions: (a) $PhB(OH)_2$ (1.05 equiv.), DMF, rt, 8 h; (b) acetone/2,2-dimethoxypropane = 1:1, TsOH (0.1 equiv.), rt, 12 h, 91% from **1**; (c) Et_3SiCl (2 equiv.), DMAP (0.1 equiv.), imidazole (3 equiv.), CH_2Cl_2, rt, 8 h, 91%; (d) NaOH, H_2O_2, 98%; (e) $POCl_3$ (8 equiv.), pyridine (20 equiv.), dry THF, rt, 8 h; (f) 1 M HCl, THF, rt, 12 h, 83% from **6**; (g) THF/H_2O, $NaHCO_3$.

The *in vitro* hypoglycemic activity of phosphoric acid **2** was tested by the glucose consumption study of HepG2 cells (Table 1) using an assay developed by Chen *et al.* [6b]. The glucose consumption of HepG2 cells increased by more than 500% in the presence of phosphoric acid **2** at a concentration range between 2×10^{-7} and 2×10^{-9} mol/l, while it increased by less than 15% in the presence of 20-hydroxylecdysone **1** at the same concentrations. At a concentration of 2×10^{-9} mol/l, phosphoric acid **2** was still active, causing a maximum increase in glucose consumption of more than 500%, whereas 20-hydroxylecdysone (**1**) was inactive at this concentration. We concluded that the synthetic phosphoric acid **2** exhibited much higher *in vitro* hypoglycemic activity than the natural 20-hydroxylecdysone (**1**), probably due to the great improvement in water solubility.

In summary, we designed and successfully synthesized a novel phosphoric acid **2** and its sodium salt **3** from the natural 20-hydroxylecdysone **1** in six steps with 67% overall yield. Subsequent biological tests demonstrated that the synthetic phosphoric acid **2** exhibited at least 40-fold more potent hypoglycemic activity than the

Table 1. Glucose lowering effects of **1** and **2**.

Sample	Concentration (M) of tested sample	GC[a] (mM) ($\bar{x} \pm s$, $n = 8$)	GC increment (mM)
Blank		1.32 ± 0.17	ND
1	2×10^{-7}	1.45 ± 0.28	0.13
1	2×10^{-8}	1.38 ± 0.11	0.06
1	2×10^{-9}	1.30 ± 0.23	ND
2	2×10^{-7}	6.96 ± 0.23	5.64
2	2×10^{-8}	6.51 ± 0.12	5.19
2	2×10^{-9}	6.46 ± 0.19	5.14

Notes: ND, not detectable.
[a] Glucose consumption.

parent compound **1**, based on tests of glucose consumption in HepG2 cells. The preliminary results indicate that **2** is an attractive candidate for development of an antidiabetic drug.

3. Experimental

3.1 General experimental procedures

All new compounds gave satisfactory spectroscopic analyses (^{1}H, ^{13}C NMR, and HR-MS). IR spectra were recorded on a FT-IR spectrometer. NMR spectra were recorded on a Bruker AC-E 200 MHz and Varian Mercury 400 MHz spectrometer. HR-MS spectra were obtained by the FAB-MS. All commercially available reagents were used without further purification. All solvents were dried and distilled before use: THF and Et_2O were distilled from sodium/benzophenone ketyl; dichloromethane was distilled from calcium hydride; methanol was distilled from Mg/I_2; $CHCl_3$ was distilled from P_2O_5. Chromatography was conducted using 200–300 mesh silica gel.

3.2 Preparation and characterization of new compounds

3.2.1 Preparation of ***4***

A solution of 20-hydroxylecdysone **1** (1.5 g, 3.21 mmol) and phenylboronic acid (412 mg, 3.38 mmol) in 20 ml of DMF was stirred at room temperature for 8 h. To the above solution, 40 ml of brine was added and the mixture was diluted with 150 ml of EtOAc. The organic layer was washed with brine (50 ml ×3), dried over sodium sulfate and concentrated *in vacuo* to get a white solid which was used directly for the next step.

The above crude product and TsOH (55 mg, 0.32 mmol) were dissolved in a 1:1 mixture of acetone and 2,2-dimethoxypropane (40 ml). After being stirred at room temperature for 12 h, the mixture was added with saturated $NaHCO_3$ (10 ml). The resulting mixture was concentrated and then diluted with EtOAc (150 ml). The organic layer was washed with brine (50 ml ×3), dried over sodium sulfate, and concentrated. The crude residue was purified by column chromatography (50% EtOAc/petroleum ether) to give **4** (1.774 g, 91%) as a colorless foam. Mp 215–224°C; $[\alpha]_D^{20} + 35$ ($c = 0.99$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, $J = 6.8$ Hz, 2H), 7.43 (t, $J = 6.8$ Hz, 1H), 7.35 (d, $J = 6.8$ Hz, 2H), 5.78 (s, 1H), 5.26 (s, 1H), 4.20–4.12 (m, 3H), 2.82 (s, 1H), 2.36–2.32 (m, 2H), 2.11–1.58 (m, 16H), 1.46 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.1, 163.7, 134.7, 131.3, 127.7, 121.2, 108.2, 86.2, 85.3, 84.5, 71.9, 71.5, 70.4, 51.3, 50.7, 47.2, 41.0, 37.7, 37.4, 34.2,

31.1, 30.7, 29.4, 29.3, 28.4, 26.5, 26.4, 25.9, 23.4, 22.4, 21.1, 20.3, 16.8; HR-FAB-MS: *m/z* 606.3701 $[M + H]^+$ (calcd for $C_{36}H_{51}BO_7$, 606.3728).

3.2.2 Preparation of **5**

To a solution of **4** (1.95 g, 3.21 mmol), imidazole (656 mg, 9.63 mmol) and DMAP (40 mg, 0.33 mmol) in CH_2Cl_2 (40 ml) was added to TESCl (1.13 ml, 6.43 mmol) dropwise at room temperature. The reaction mixture was stirred for 4 h and then diluted with EtOAc (150 ml) and washed with brine (50 ml × 3), dried over sodium sulfate, and concentrated. The crude residue was purified by column chromatography (17% EtOAc/petroleum ether) to give **5** (2.11 g, 91%) as a colorless foam. $[\alpha]_D^{20} + 30.4$ ($c = 0.97$, CH_2Cl_2). IR (KBr) ν_{max}: 2959, 1660, 1356, 1242, 1056 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, $J = 7.2$ Hz, 2H), 7.46 (t, $J = 7.2$ Hz, 1H), 7.36 (t, $J = 7.2$ Hz, 2H), 5.82 (s, 1H), 4.26–4.21 (m, 2H), 4.13–4.09 (m, 1H), 2.84 (t, $J = 2.8$ Hz, 1H), 2.38–2.34 (m, 2H), 2.11–2.04 (m, 3H), 2.04–1.81 (m, 7H), 1.78–1.57 (m, 4H), 1.54–1.45 (m, 1H), 1.50 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H), 1.27–1.22 (m, 1H), 1.26 (s, 3H), 1.25 (s, 3H), 1.00 (s, 3H), 0.96 (t, $J = 8.0$ Hz, 9H), 0.95 (s, 3H), 0.59 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 202.6, 162.9, 134.8, 131.3, 127.9, 127.7, 121.5, 108.3, 86.2, 85.4, 85.0, 73.0, 72.1, 71.6, 51.9, 50.8, 47.3, 42.1, 37.8, 37.6, 34.5, 31.6, 30.9, 30.4, 29.7, 28.5, 26.7, 26.4, 23.6, 22.5, 21.2, 20.5, 17.0, 14.2, 7.1, 6.8; HR-FAB-MS: *m/z* 743.4452 $[M + Na]^+$ (calcd for $C_{42}H_{65}BNaO_7Si$, 743.4490).

3.2.3 Preparation of **6**

To a solution of **5** (2.23 g, 3.00 mmol) in 30 ml of CH_2Cl_2, 1 M NaOH (20 ml) and 30% H_2O_2 (10 ml) were added at room temperature. The resulting mixture was stirred for 30 min and then diluted with EtOAc (100 ml). The organic layer was washed with brine (50 ml × 3), dried over sodium sulfate, and concentrated. The crude residue was purified by column chromatography (25% EtOAc/petroleum ether) to give **6** (1.88 g, 98%) as a colorless foam. $[\alpha]_D^{20} + 23.4$ ($c = 1.915$, CH_2Cl_2); IR (KBr) ν_{max}: 2960, 1659, 1378, 1239, 1056 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 5.82 (d, $J = 0.8$ Hz, 1H), 4.27–4.20 (m, 2H), 3.42 (d, $J = 10.4$ Hz), 2.81 (t, $J = 8.4$ Hz, 1H), 2.36–2.30 (m, 2H), 2.11–2.03 (m, 4H), 1.98–1.93 (dd, $J = 14.4$, 5.6 Hz, 1H), 1.88–1.81 (m, 2H), 1.78–1.66 (m, 6H), 1.62–1.46 (m, 2H), 1.48 (s, 3H), 1.37–1.32 (m, 1H), 1.32 (s, 3H), 0.98 (s, 3H), 0.95 (t, $J = 8.0$ Hz, 9H), 0.86 (s, 3H), 0.59 (q, $J = 8.0$ Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 202.7, 163.3, 121.4, 108.3, 84.9, 76.6, 73.9, 72.2, 71.6, 50.8, 49.1, 47.6, 42.1, 37.8, 37.6, 34.5, 31.8, 31.2, 30.2, 29.7, 28.5, 26.7, 26.4, 26.1, 23.6, 20.8, 20.5, 20.4, 17.4, 7.1, 6.6; HR-FAB-MS: *m/z* 657.4131 $[M + Na]^+$ (calcd for $C_{36}H_{62}NaO_7Si$, 657.4163).

3.2.4 Preparation of 20-hydroxylecdysono-20,22-phosphonic acid **2**

To a solution of **6** (1.34 g, 2.11 mmol) and pyridine (3.4 ml) in 30 ml of dry THF, $POCl_3$ (0.96 ml, 10.5 mmol) was added dropwise at 0°C. The reaction mixture was stirred at room temperature for 8 h and then quenched by adding H_2O (2 ml) carefully. The mixture was diluted with EtOAc (150 ml), washed with brine (50 ml × 1), dried over sodium sulfate, and concentrated. The residue was dissolved in 30 ml of THF, and 1 M HCl (10 ml) was added. The reaction mixture at room temperature for 12 h and then concentrated. The crude residue was purified by column chromatography on C18 silica gel (20% MeOH/H_2O) to give **2** (891 mg, 83%) as a white powder. Purity was determined to be 98.7% by reverse phase HPLC analysis using a gradient of

methanol/0.01 M sodium citrate (pH 7.0, adjusted with 0.01 M citric acid), and the mobile phase increased linearly from 10% MeOH at 0 min to 50% MeOH at 30 min. Mp 164–177°C; $[\alpha]_D^{20}$ + 62.6 (c = 0.89, 95% EtOH); IR (KBr) ν_{max} 2966, 1653, 1384, 1227 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 5.81 (s, 1H), 4.24 (s, 1H), 3.94 (s, 1H), 3.84–3.81 (m, 1H), 3.16–3.12 (m, 1H), 2.40–2.36 (m, 2H), 2.17–2.10 (m, 2H), 2.02–1.88 (m, 2H), 1.84–1.62 (m, 10 H), 1.54–1.40 (m, 2H), 1.46 (s, 3H), 1.20 (s, 3H), 1.19 (s, 3H), 0.96 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD) δ 206.3, 166.9, 122.4, 91.8, 86.7, 85.0, 70.8, 68.7, 68.5, 51.8, 51.0, 50.9, 41.5, 39.2, 37.4, 35.1, 32.8, 32.1, 31.6, 29.6, 28.9, 25.9, 25.6, 24.4, 22.3, 21.4, 17.4; ^{31}P NMR δ 15.8 referenced to external H_3PO_4; HR-FAB-MS: *m/z* 565.2520 $[M + Na]^+$ (calcd for $C_{27}H_{43}NaO_9P$, 565.2542).

3.2.5 *Preparation of sodium salt* ***3***

To a solution of phosphoric acid **2** in H_2O, saturated sodium bicarbonate was added until the pH of the solution reached 7.5. After concentration, the residue was purified by column chromatography on C18 silica gel (20% MeOH/H_2O) to give sodium salt **3** as a white powder. $[\alpha]_D^{20}$ + 52.1 (c = 0.99, 95% EtOH); mp 173–185°C; IR (KBr) ν_{max}: 2966, 1651, 1382, 1206 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 5.80 (s, 1H), 4.13 (dd, J = 9.2, 2.8 Hz, 1H), 3.95 (s, 1H), 3.84–3.81 (m, 1H), 3.17–3.13 (m, 1H), 2.40–2.33 (m, 2H), 2.20–2.11 (m, 2H), 1.99–1.58 (m, 12 H), 1.51–1.40 (m, 2H), 1.42 (s, 3H), 1.20 (s, 3H), 1.18 (s, 3H), 0.96 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD): δ 206.3, 167.3, 122.2, 88.8, 85.1, 84.6, 70.9, 68.6, 68.4, 51.7, 50.9, 50.8, 42.0, 39.2, 37.3, 35.0, 32.8, 32.1, 31.7, 29.7, 28.8, 25.9, 25.8, 24.5, 22.4, 21.5, 17.5; ^{31}P NMR δ 14.3 referenced to external H_3PO_4; HR-FAB-MS: *m/z* 565.2540 $[M + H]^+$ (calcd for $C_{27}H_{43}NaO_9P$, 565.2542).

Acknowledgements

This work was financially supported by grants from the Natural Science Foundation of China (20825207, 20972083, and 21021001), the Program for Changjiang Scholars and Innovative Research Team in University (IRT0846), the National Basic Research Program of China (973 program, 2010CB833200), the National Science and Technology Major Project of China (2009ZX09310-002), and the State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry.

References

[1] (a) J. Colombani, L. Bianchini, S. Layalle, E. Pondeville, C. Dauphin-Villemant, Ch. Antoniewski, C. Carre, S. Noselli, and P. Leopold, *Science* **310**, 667 (2005). (b) R. Lafont and L. Dinan, *J. Insect. Sci.* **3**, 7 (2003). (c) L. Dinan, *Phytochemistry* **57**, 325 (2001). (d) J. Koolman, *Ecdysone: From Chemistry to Mode of Action* (Thiem Verlag, Stuttgart, 1989).

[2] E.A. Schmelz, R.J. Grebenok, T.E. Ohnmeiss, and W.S. Bowers, *Arch. Insect. Biochem. Physiol.* **51**, 204 (2002) and references cited therein.

[3] V.N. Mironova, T. Kholodova, T.F. Skachkova, and I. Bondar, *Vopr. Med. Khim.* **28**, 101 (1982).

[4] (a) A.G. Kurmukov and O.A. Ermishina, *Farmakol Toksikol.* **54**, 27 (1991). (b) X. Wu, J. Jin, and F.J. Si, *China J. Chinese Mater. Med.* **26**, 332 (2001).

[5] T. Yoshida, T. Otaka, M. Uchiyama, and S. Ogawa, *Biochem. Pharmacol.* **20**, 3263 (1971).

[6] (a) P. Kizelsztein, D. Govorko, S. Komarnytsky, A. Evans, Z. Wang, W.T. Cefalu, and H. Raskin, *Am. J. Physiol-Endoc. M.* **296**, E433 (2009). (b) Q. Chen, Y.P. Xia, and Z.Y. Qiu, *Life Sci.* **78**, 1108 (2006). (c) Q. Chen, Y.P. Xia, and Z.Y. Qiu, *Chinese Pharm. Bull.* **22**, 460 (2006). (d) M. Wessner, B. Champion, J.P. Girault, M. Kaouadji, B. Saidi, and R. Lafront, *Ajuga iva. Phytochem.* **31**, 3785 (1992).

[7] (a) RU Patent No. 2 337 698 (2008). (b) CN Patent No. 1 280 010 (2008).

[8] R. Bergamasco and D.H.S. Horn, *The Biological Activities of Ecdysteroids and Ecdysteroid Analogs* (Elsevier, Amsterdam, 1980), pp. 299–324.

[9] A. Suksamrarn, P. Pattanaprateep, T. Tanachatchairatana, W. Haritakun,

B. Yingyongnarongkul, and N. Chimnoi, *Insect Biochem. Mol. Biol.* **32**, 193 (2002).

[10] A number of natural ecdysteroid phosphates, including 20-hydroxyecdysono-22-phosphate, have been isolated, see: (a) R.E. Isaac and H.H. Rees, *Biochem. J.* **221**, 459 (1984). (b) R.E. Isaac, M.E. Rose, H.H. Rees, and T.W. Goodwin, *Biochem. J.* **213**, 533 (1983).

[11] (a) N.V. Kovganko, Z.N. Kashkan, Y.G. Chernov, S.K. Ananich, S.N. Sokolov, and V.L. Survilo, *Chem. Nat. Compd* **39**, 411 (2003). (b) N.K. Politova, L.A. Kovler, V.V. Volodin, V.G. Luksha, and E.A. Pshunetleva, *Khimiya Rastitel'nogo Syr'ya* **2**, 69 (2001).