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SYNTHESIS AND BIOACTIVITY OF BRASSINOSTEROID BORATES

Abstract. A regioselective synthesis of boric acid esters derived from 24-epibrassinolide and 24-epicastasterone has been developed. The esterification proceeds under mild conditions with high efficiency, yielding the corresponding 22,23-borates of steroidal phytohormones as the sole products. Their plant growth-stimulating activity was evaluated using spring wheat (*Triticum aestivum* L.) as a model system. The compounds were applied *via* seed soaking in solutions at concentrations of 10^{-7} and 10^{-9} M for 24 hours. Germination rates were assessed after 7 days, while seedling height, root length, and fresh weight of both seedlings and roots were measured 10 days after the start of the treatment. The synthesized conjugates were found to enhance seed germination and stimulate the growth of above-ground parts (by 10–15 % relative to the control), as well as increase biomass accumulation (by 13–23 %) and root weight (by 8–22 %). These novel brassinosteroid derivatives (borates) exhibited higher plant growth-stimulating activity than the native hormones themselves.

Keywords: brassinosteroids, 24-epibrassinolide, 24-epicastasterone, borates, synthesis, *Triticum aestivum*, seed germination, plant growth-stimulating activity

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СИНТЕЗ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ БОРАТОВ БРАССИНОСТЕРОИДОВ

Аннотация. Разработан региоселективный синтез эфиров борной кислоты на основе 24-эпибрасинолида и 24-эпикастастерона. Реакция этерификации протекает в мягких условиях с высоким выходом, приводя к образованию соответствующих 22,23-боратов стероидных фитогормонов в качестве единственных продуктов. Их ростостимулирующая активность была оценена с использованием яровой пшеницы (*Triticum aestivum* L.) в качестве модельной системы. Соединения вносили путем замачивания семян в растворах с концентрациями 10^{-7} и 10^{-9} М в течение 24 ч. Всхожесть оценивали через 7 дней, а высоту проростков, длину корней и сырую массу как проростков, так и корней измеряли через 10 дней после начала обработки. Было обнаружено, что синтезированные конъюгаты повышают всхожесть семян и стимулируют рост надземных частей (на 10–15 % по отношению к контролю), а также увеличивают накопление биомассы (на 13–23 %) и массу корней (на 8–22 %). Эти новые производные брасиностероидов (бораты) проявили более высокую ростостимулирующую активность, чем сами нативные гормоны.

Ключевые слова: брасиностероиды, 24-эпибрасинолид, 24-эпикастастерон, бораты, синтез, *Triticum aestivum*, всхожесть семян, ростостимулирующая активность

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Introduction. Phytohormonal steroids (brassinosteroids, BS) are widespread in nature – they are characteristic of all, or the vast majority, of plant species and are known as natural plant hormones with growth-stimulating and adaptogenic effects [1]. Boron is one of the most important trace elements necessary for optimal plant development [2]. It is essential for cell division, protein synthesis, photosynthesis, as well as successful flowering and fruiting. It also increases resistance to diseases, drought, and frost.

In our previous work [3], it was shown that the modification of steroid phytohormones with acids of various structures (5-aminolevulinic, indole-3-acetic, salicylic, succinic, sulfuric) leads to ester derivatives that, in terms of growth-stimulating activity, are generally not inferior to, and even exceed, the original brassinosteroids. It should be particularly noted that the BS modified with acids performed well in experiments studying the protection of plants from various stressors. For instance, the auxin derivative has a positive effect on the growth and development of *Arabidopsis thaliana* and *Triticum aestivum* plants under salt stress conditions [4]. The synthesized 2-monosalicylates of BS had a positive effect on plant resistance to various types of stress: heat and salt stress [5–7], and biotic stress (stressor – the phytopathogenic fungus *Helminthosporium teres* Sacc.) [8; 9]. The tetrahemisuccinate of 24-epibrassinolide mitigates the negative effects of salt stress (150 mM NaCl) on rapeseed plants [10].

It seemed of interest to investigate the growth-stimulating activity of compounds that would contain a brassinosteroid moiety and a boron component. The aim of the present work is to synthesize boron derivatives of 24-epibrassinolide (EBI) and 24-epicastasterone (EBk) and to study their growth-stimulating activity.

Experimental. NMR spectra were recorded on a Bruker BioSpin AVANCE 500 spectrometer (500 MHz) in C_5D_5N . 1H and ^{13}C chemical shifts were referred to residual C_5H_5N (δ 8.74 ppm) and C_5D_5N (δ 150.35), respectively. Epicastasterone (**1**) and epibrassinolide (**2**) were synthesized in the Laboratory of Steroid Chemistry of the Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus according to published procedures [11]. The progress of the reactions was monitored by TLC on Merck 60 F₂₅₄ plates. The purification of the synthesized compounds was carried out by column chromatography on Merck 60 silica gel (0.063–0.2 mm).

(22R,23R,24R)-2 α ,3 α ,22,23-Tetrahydroxy-24-methyl-5 α -cholestan-6-one 22,23-borate (3). To a solution of epicastasterone (**1**) (100 mg, 0.22 mmol) in tetrahydrofuran (2 mL), boric acid (14 mg, 0.22 mmol) was added. The mixture was stirred at room temperature for 15 min, then solvent was evaporated *in vacuo*. The residue was chromatographed on SiO₂ (eluent: chloroform–ethanol = 30 : 1 → 10 : 1) to give ester **3** (101 mg, 95 %) as an amorphous solid. NMR data are shown in Table.

1H and ^{13}C NMR spectroscopic data for **3**^{a,b,c}

Position	δ , C	δ , H
1	41.7	2.05 m
2	69.0	4.09 ddd (11.6, 5.2, 2.9)
3	69.5	4.46 q (3.0)
4	28.4	2.02 m; 2.34 m
5	52.0	3.16 dd (12.6, 3.2)
6	212.4	
7	47.4	2.02 m; 2.38 m
8	38.3	1.76 m
9	57.0	1.16 m
10	43.4	
11	22.0	1.25 m; 1.61 m
12	40.0	1.11 m; 1.90 m
13	43.1	
14	54.3	1.35 m
15	24.6	1.04 m; 1.48 m
16	28.7	1.37 m; 2.09 m
17	53.4	1.75 m
18	12.4	0.66 s
19	14.3	0.85 s
20	42.5	1.45 m
21	12.5	1.13 d (6.6)
22	82.3	4.33 d (4.7)
23	81.6	3.97 dd (9.1, 4.8)
24	45.4	1.60 m
25	28.0	2.26 pd (6.9, 3.7)
26	21.7	0.91 d (6.9)
27	17.0	0.91 d (6.9)
28	9.7	0.75 d (6.8)

Notes: ^a – NMR chemical shifts (δ) are from spectra obtained in C_5D_5N solution; ^b – assigned by DEPT, COSY, NOESY, HSQC, and HMBC experiments; ^c – J values (in Hz) in parentheses.

(22*R*,23*R*,24*R*)-2 α ,3 α ,22,23-Tetrahydroxy-B-homo-7-oxa-24-methyl-5 α -cholestan-6-one 22,23-borate (4). The title compound was synthesized starting from epibrassinolide (2) and boric acid in 93 % yield as an amorphous solid according to the procedure described for the preparation of 3. ¹H NMR (500 MHz, C₅D₅N) δ 4.37 (p, J = 2.4 Hz, 1H), 4.23 (d, J = 4.7 Hz, 1H), 4.09 – 3.93 (m, 3H), 3.88 (dd, J = 9.1, 4.7 Hz, 1H), 3.80 (q, J = 7.0 Hz, 1H), 3.55 (dd, J = 12.2, 4.4 Hz, 1H), 2.45 (ddd, J = 14.5, 12.0, 2.2 Hz, 1H), 2.25 (dt, J = 14.9, 4.2 Hz, 1H), 2.17 (pd, J = 6.9, 3.8 Hz, 1H), 2.07 (d, J = 8.3 Hz, 2H), 1.99 (dtd, J = 12.9, 9.5, 6.0 Hz, 1H), 1.02 (d, J = 6.5 Hz, 3H), 0.98 (s, 3H), 0.83 (d, J = 6.9 Hz, 6H), 0.67 (d, J = 6.8 Hz, 3H), 0.56 (s, 3H). ¹³C NMR (126 MHz, C₅D₅N) δ 177.2, 82.2, 81.5, 70.7, 69.2, 68.9, 58.7, 53.3, 51.7, 45.3, 43.2, 43.0, 42.4, 42.2, 40.1 (\times 2), 39.0, 33.6, 28.6, 27.9, 25.4, 22.9, 21.6, 16.9, 16.4, 12.4, 12.1, 9.7.

Biological studies. Spring wheat (*Triticum aestivum*) of the Belarusian variety Madonna was chosen as the object of the study. In laboratory experiments, seeds were soaked in solutions of phytohormonal steroids and their borates (solutions of 10⁻⁹ and 10⁻⁷ M were used). Water was used as a control. 50 seeds were placed in a Petri dish and filled with 10 ml of the corresponding solution. After 18 hours, the solutions were drained, and the dried seeds were placed in Petri dishes on filter paper moistened with distilled water. The seeds were germinated in a thermostat at 20 °C, and then under an LED lamp (red-blue light) DBP-03-14-820 with a power of 14 W, with constant moistening of the bed with distilled water. The experiments were performed in 4-fold replication. According to GOST 12038-84, germination was assessed after 7 days and after 10 days, measurements of sprout height and root length, as well as fresh weight of sprouts and roots of control and experimental variants, were taken. The figures show the mean values and their standard deviations. The significance of differences was calculated using Student's *t*-test. Differences are considered significant at $p \leq 0.05$.

Results and Discussion. Epicastasterone and epibrassinolide each contain two vicinal diol groups, each of which can, in principle, react with derivatives of boric acid to form corresponding cyclic borates (boronates). Thus, the reaction of methylboronic acid with brassinosteroids leads to 2,3,22,23-bis(methylboronates), which have been widely used in the analysis of this group of phytohormones [12]. A similar reaction with boric acid proceeds regioselectively at the diol group of the side chain, which we utilized for the synthesis of steroidal C-22 aldehydes [13]. In that case, the intermediately formed boric acid derivatives were not isolated. In the present work, the 22,23-borates of epicastasterone and epibrassinolide have been isolated in pure form for the first time with the aim of studying their biological activity. Both compounds were obtained in nearly quantitative yields by reacting equimolar amounts of epicastasterone (1) or epibrassinolide (2) with boric acid in tetrahydrofuran (Fig. 1). A complete assignment of the signals in the NMR spectrum was made for compound 3. The presence of signals at δ 4.09

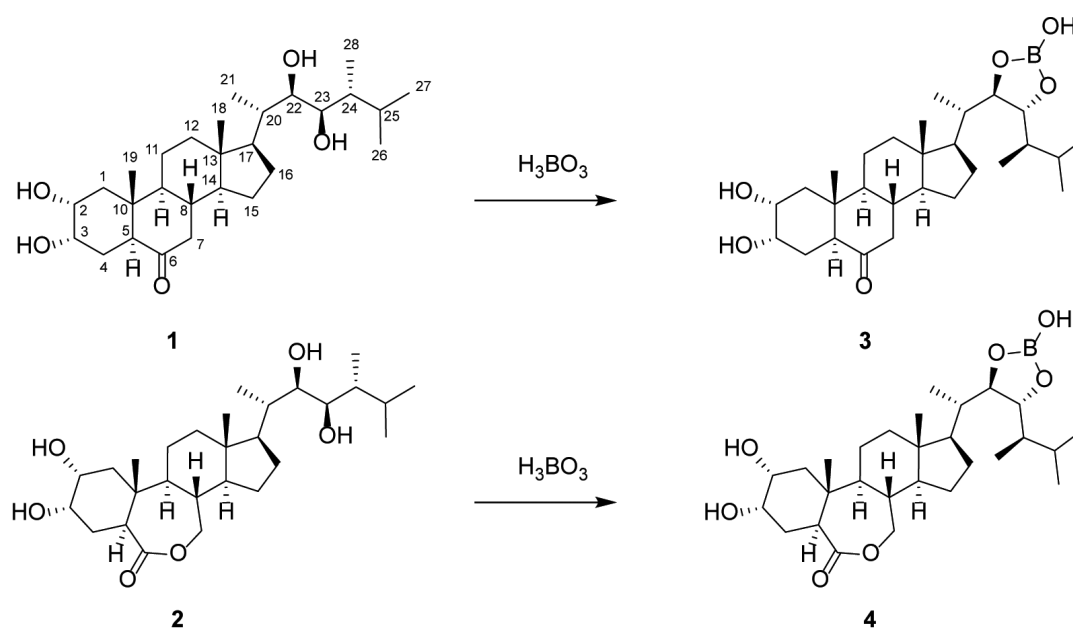


Fig. 1. Synthesis of 22,23-borates 3 and 4

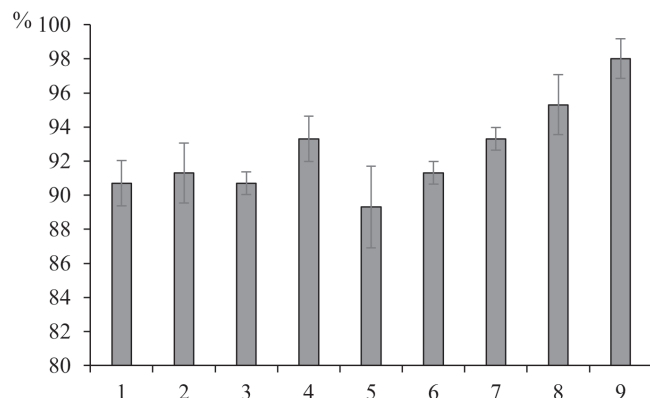


Fig. 2. Effect of phytohormonal steroids and their conjugates with boric acid on the germination of spring wheat seeds: 1 – control, water; 2 – EBI, 10^{-9} M; 3 – EBI, 10^{-7} M; 4 – EBk, 10^{-9} M; 5 – EBk, 10^{-7} M; 6 – borate 4, 10^{-9} M; 7 – borate 4, 10^{-7} M; 8 – borate 3, 10^{-9} M; 9 – borate 3, 10^{-7} M

Fig. 2 presents data on the effect of phytohormonal steroids and their conjugates with boric acid on the germination of spring wheat seeds. The obtained results demonstrate a significant effectiveness of the conjugates, with the highest activity observed at the lower concentration of 10^{-9} M. It is noteworthy that the effect of the boron-containing BS derivatives is generally more effective not only compared to the control, but also compared to the effect of the individual BS.

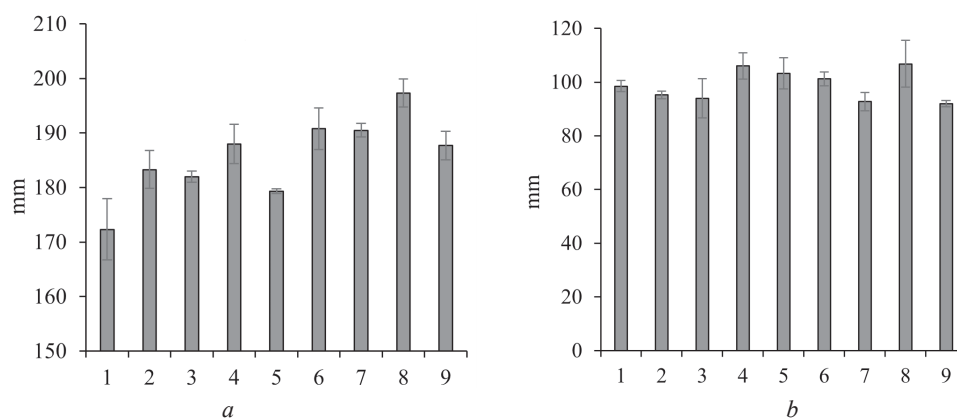


Fig. 3. Effect of phytohormonal steroids and their conjugates with boric acid on sprout height (a) and root length (b) of spring wheat: 1 – control, water; 2 – EBI, 10^{-9} M; 3 – EBI, 10^{-7} M; 4 – EBk, 10^{-9} M; 5 – EBk, 10^{-7} M; 6 – borate 4, 10^{-9} M; 7 – borate 4, 10^{-7} M; 8 – borate 3, 10^{-9} M; 9 – borate 3, 10^{-7} M

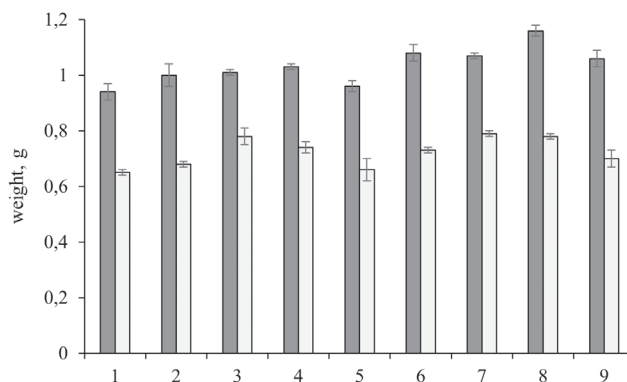


Fig. 4. Effect of phytohormonal steroids and their conjugates with boric acid on the fresh weight of spring wheat sprouts (■) and roots (□): 1 – control, water; 2 – EBI, 10^{-9} M; 3 – EBI, 10^{-7} M; 4 – EBk, 10^{-9} M; 5 – EBk, 10^{-7} M; 6 – borate 4, 10^{-9} M; 7 – borate 4, 10^{-7} M; 8 – borate 3, 10^{-9} M; 9 – borate 3, 10^{-7} M

(ddd, $J = 11.6, 5.2, 2.9$ Hz) and δ 4.46 (q, $J = 3.0$ Hz), corresponding to the methine protons at C-2 and C-3 respectively, confirms the correctness of the structural assignment, since similar signals are present in the spectrum of the starting epicastasterone (1).

A key factor explaining the high regioselectivity of this reaction may be the differing stabilities of the 2,3- and 22,23-boronate esters. The stability of *cis*-diol borates is known to depend critically on the orientation of the OH groups [14]. In our case it leads to the unexpected result where boric acid condenses exclusively with the 22,23 *cis*-diol of the side chain, even though these hydroxyl groups are known to be less reactive than those of the A-ring [1].

The results of the effect of the synthesized compounds on the morphometric parameters of wheat seedlings are shown in Fig. 3. The sprout height increased by 11 % compared to the control in the case of 24-epibrassinolide borate (4) and by 10–15 % with the application of 24-epicastasterone borate (3), while the root length increased only at a concentration of 10^{-9} M, and some inhibition of growth was observed at a concentration of 10^{-7} M.

Fresh weight parameters (Fig. 4) for both sprouts and roots at the studied concentrations significantly exceeded the control variant – the increase for sprouts amounted to 113–123 %, and for roots, 108–122 %. These values for the borates were also higher than those obtained using the individual BS in all cases (except for EBI at a concentration of 10^{-7} M).

Conclusions. Synthesis of boric acid esters with 24-epibrassinolide and 24-epicastasterone was carried out, and their growth-stimulating effect on spring wheat plants (*Triticum aestivum* L.) of the variety Madonna was studied for the first time. It was found that the obtained conjugates of brassinosteroids with boric acid exhibit notable growth-stimulating activity, exceeding that of the control in terms of growth parameters (seed germination, sprout and root length, weight of above-ground parts and roots). For all parameters, the concentration of 10^{-9} M proved to be the most effective.

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