Chemistry of Natural Compounds, Vol. 30, No. 3, 1994

SYNTHESIS OF (24R)-HOMOBRASSINOLIDE

V. A. Khripach, V. N. Zhabinskii, and V. K. Ol'khovik

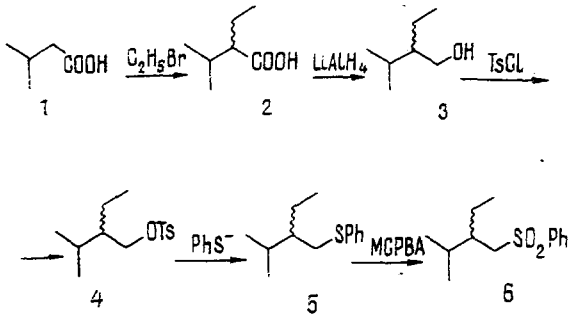
UDC 547.92.057

The synthesis from stigmasterol of C-29 brassinosteroids containing an (R)-ethyl group at C24 — (24R)-homocastasterone and (24R)-homobrassinolide — is described. The structure of the carbon skeleton of the side-chain was achieved by condensing a C-22 aldehyde with the appropriate sulfone

It is known that plant sources contain a rich set of sterols [1]. While being important structural elements of membranes, sterols are also the initial compounds in the course of the biosynthesis of a whole series of steroids fulfilling hormonal, protective, or other, functions. It is obvious that this applies in full measure to a new class of plant hormones — the brassinosteroids [2]. About 30 representatives of this class of compounds have been detected, but hitherto no brassinosteroids with an (R)-ethyl substituent at C-24 have become known, even though possible biosynthetic precursors of them — poriferasterol, clionasterol — are widespread in the vegetable kingdom. A possible reason for this may be an imperfection of analytical methods, since brassinosteroids are present in plants in extremely small amounts ($10^{-7}-10^{-12}$% and less) [3]. The search for new compounds in natural sources would be considerably facilitated by the existence of authentic samples obtained synthetically.

The aim of the present work was the synthesis of (24R)-homobrassinolide and (24R)-homocastasterone. A single synthesis of these compounds, from the scarce sterol poriferasterol, has been described in the literature [4]. We have now developed a method of synthesizing (24R)-brassinosteroids from a more accessible raw material — stigmasterol.

A key intermediate — the aldehyde (**7**) — was obtained from stigmasterol in five stages. We have used it previously in the synthesis of norbrassinolide [5] and brassinolide [6]. The construction of the side-chains of the desired compounds was carried out by the condensation of the 22-aldehyde (**7**) with the sulfone (**6**), which was synthesized from isovaleric acid (**1**).



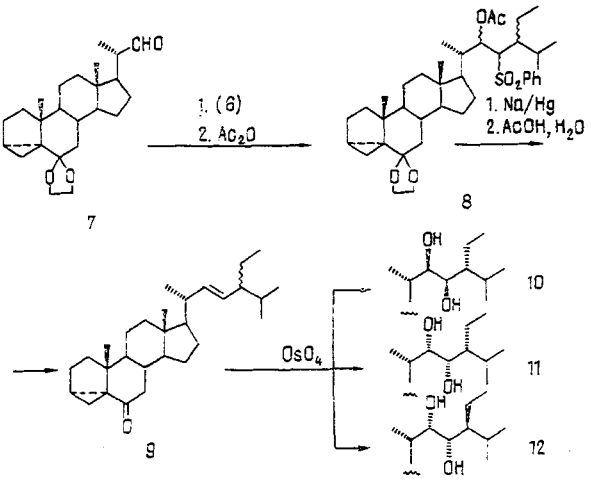
The introduction of an ethyl group was achieved by treating with ethyl bromide the carbanion obtained from acid (**1**) and butyllithium. Reduction of the acid (**2**) with $LiAlH_4$ gave the alcohol (**3**), which was converted into the sulfide (**5**) via the tosylate (**4**). Oxidation of the sulfide (**5**) with *m*-chloroperbenzoic acid gave the sulfone (**6**). The structure of compound (**6**) was confirmed by its spectral characteristics. Thus, in the IR spectrum we observed absorption bands at 1150 and 1310 cm^{-1}, corresponding to the stretching vibrations of a S=O bond. The mass spectrum contained the peak of the molecular ion with *m/z* 240 and also peaks with *m/z* 142 ($PhSO_2$) and 99, 98 ($M^+ - PhSO_2$; $M^+ - PhSO_2H$). In the PMR spectrum all the signals of the methyl, methylene, and methine protons of this molecule were well defined. Condensation of the aldehyde

Institute of Organic Chemistry, Belarus Academy of Sciences, Minsk. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 385-391, May-June, 1994. Original article submitted July 14 , 1993.

0009-3130/94/3003-0352$12.50

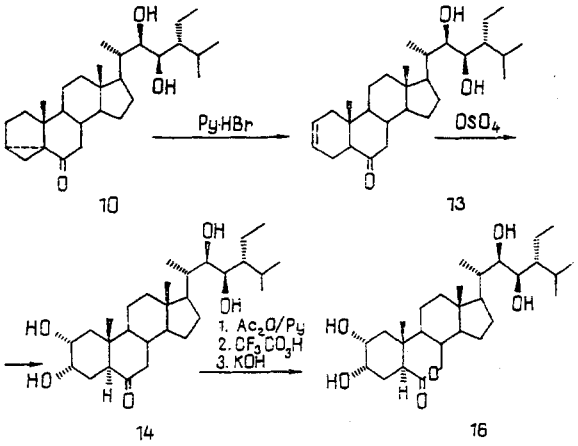
(7) with the α-sulfonyl carbanion obtained from sulfone (6) and butyllithium followed by treatment with acetic anhydride led to the acetoxysulfone (8). Reductive treatment of the latter with sodium amalgam in a mixture of methanol and ethyl acetate and elimination of the dioxolane protection gave the 22-ene-ketone (9) with an overall yield of 68.8%.

The subsequent functionalization of the molecule was effected by the successive introduction of 22,23- and 2α,3α-diol groupings and the lactonization of ring B.



The hydroxylation of the eneketone (9) with osmium tetroxide in pyridine led to a mixture of isomeric diols, three of which, after chromatographic separation, were characterized as the diols (10), (11), and (12). Their structures were established on the basis of spectral characteristics in comparison with the literature, and, in the case of diol (12), obtained previously from stigmasterol [7], also by a direct comparsion of specimens.

Of the three diols isolated (10-12), interest in connection with the solution of the problem posed was presented by diol (10) which, like all natural brassinosteroids known at the present time, contained a 22R,23R-diol grouping.



The introduction of the Δ^2 bond was achieved in one stage by boiling compound (10) in dimethylformamide in the presence of pyridine hydrobromide. Oxidation with osmium tetroxide of the enediol (13) formed gave (24R)-ethylbrassinone (14) with a yield of 98%. The structure of the compound obtained was confirmed by a combination of physicochemical characteristics, which agreed with those given in the literature [4]. The introduction of a lactone function into ring *B* of compound (14) was effected by the Baeyer–Villiger oxidation of the acetyl derivative (15), followed by saponification and relactonization.

EXPERIMENTAL

Melting points were measured on a Kofler block. IR spectra were obtained on a UR-20 instrument in KBr tablets and in films. PMR spectra were recorded on JNM-PS-100 and WM-360 NMR spectrometers, with working frequencies of 100 and 360 MHz, respectively, in 2% solutions of $CDCl_3$ with TMS as internal standard. Mass-spectrometric characteristics were obtained on a Varian MAT-311 instrument at an energy of the ionizing radiation of 70 eV. Type LSL_{254} silica gel and Silufol-UV-254 plates were used for the analytical monitoring of the course of the reactions.

2-Ethyl-3-methylbutanoic Acid (2). A solution of butyllithium in hexane (420 ml, 1.55 N) was diluted with 200 ml of THF and it was then cooled to — 20°C and treated with 90 ml of diisopropylamine. Then a solution of 15 g of isovaleric acid in 200 ml of THF was added, the temperature was raised to — 5°C, 30 ml of hexamethyphosphorotriamide was added, and the resulting reaction mixture was kept for 30 min. It was cooled to — 30°C and a solution of ethyl bromide was slowly added, after which it was kept at room temperature for 2 h, and it was then diluted with water and evaporated. The residue was again diluted with water, and the neutral products were extracted with ether (2 × 50 ml). Then the aqueous part was acidified with hydrochloric acid and extracted with ether. The extract was dried over Na_2SO_4 and evaporated, and the residue was distilled. A fraction with bp 198-202°C was collected, and 11.6 g of compound (**2**) was isolated (60% yield). IR spectrum (cm^{-1}): 1710 (C=O). PMR spectrum (100 MHz, δ, ppm): 0.88-1.06 m (9H, 4-, 5-, 7-Me), 1.53-2.06 m (4H, C_2-H, C_3-H, C_6-H_2), 13.1 s (1H, COOH). Mass spectrum (*m/z*): 130 (M^+), 85 (M^+-COOH).

2-Ethyl-3-methylbutanol (3). With continuous stirring, a solution of 11.6 g of acid (**2**) in 50 ml of ether was added to a suspension of 5 g of $LiAlH_4$ in 100 ml of ether. The reaction mixture was kept for 1 h and was then diluted with 20% sulfuric acid and extracted with ether. The extract was dried over Na_2SO_4 and evaporated. The residue was distilled, with the collection of a fraction having bp 185-190°C and consisting of 5 g of the alcohol (**3**) (yield 50%). IR spectrum (cm^{-1}): 3350 (OH). PMR spectrum (100 MHz, δ, ppm): 0.88-1.09 m (9H, 4-, 5-, 7-Me), 1.12-1.39 m (2H, C_6-H_2), 1.55-2.16 m (2H, C_2-H, C_3-H), 3.7 m (2H, C_1-H_2). Mass spectrum (*m/z*): 116 (M^+), 85 ($M^+-CH_2 = OH^+\cdot$), 88 (M^+-Et).

2-Ethyl-3-methyl-1-(*p*-tolylsulfonyloxy)butane (4). A solution of 5 g of the alcohol (**3**) in 35 ml of pyridine was treated with 17 g of *p*-toluenesulfonyl chloride, and the reaction mixture was kept for 2 h. Then it was diluted with water and extracted with ether. The extract was evaporated, and the residue was chromatographed on a column of silica gel, with elution by ether–hexane (10:1). This gave 3.3 g of the tosylate (**4**). IR spectrum (cm^{-1}): 1600 ($Ph-CH_3$), 1360 (S=O). PMR spectrum (360 MHz, δ, ppm): 0.79 m (9H, 4-, 5-, 7-Me), 1.18-1.27 m (2H, C_6-H_2), 1.31-1.34 m (1H, C_2-H) 1.67-1.78 m (1H, C_3-H), 2.45 s (3H, $Ph-CH_3$), 3.97 dd (2H, J_1 = 6 Hz, J_2 = 2.4 Hz, C_1-H_2), 7.34-778 m (4H, Ts). Mass spectrum (*m/z*); 270 (M^+).

2-Ethyl-3-methylbutyl Phenyl Sulfide (5). Thiophenol (3.4 ml) was added to a solution of sodium methanolate in methanol prepared from 2 g of sodium in 150 ml of methyl alcohol. Then, with stirring, a solution of 3.3 g of the tosylate (**4**) in 20 ml of methanol was added. The reaction mixture was kept at room temperature for 10 h, and was diluted with water and extracted with ether. The extract was evaporated, and the residue was chromatographed on a column of silica gel, with elution by hexane–ether (10:1). This led to the isolation of 2.1 g of compound (**5**). Yield 80%. IR spectrum (cm^{-1}) 1585, 1480, 1438, 1385, 1370, 990, 930. PMR spectrum (360 MHz, δ, ppm): 0.88 m (9H, 4-, 5-, 7-Me), 1.33-1.50 m (3H, C_2-H, C_6-H), 1.90 m (1H, C_3-H), 2.81 dd (1H, J_1 = 12.6 Hz, J_2 = 6 Hz, C_1-H), 3.95 dd (1H, J_1 = 12.6 Hz, J_2 = 6 Hz C_1-H), 7.08 tt (1H, J_1 = 7.2 Hz, J_2 = 1.2 Hz, Ph), 7.18–7.30 m (4H, Ph). Mass spectrum (*m/z*): 208 (M^+), 99 (M^+-PhS).

2-Ethyl-3-methylbutyl Phenyl Sulphone (6). With ice cooling, 6.4 g of *m*-chloroperbenzoic acid was added to a solution of 1.9 g of sulfide (**5**) in 160 ml of chloroform. The mixture was stirred at room temperature for 2 h and was then diluted with a 25% solution of ammonia (100 ml) and extracted with chloroform. The extract was filtered through a layer of alumina and was evaporated, and the residue was chromatographed on a column of silica gel with elution by ether–hexane (20:1). The resulting yield of compound (**6**) was 2.7 g (95%). IR spectrum (cm^{-1}): 1310, 1320 (S=O), 1150 (S=O). PMR spectrum (360 MHz, δ, ppm): 0.75 d (3H, J = 2.4, 4-Me), 0.77 d (3H, J = 2.4 Hz, 5-Me), 0.82 t (3H, J = 7.2 Hz 7-Me), 1.35 m (2H, C_6-H_2), 1.75 m (1H, C_3-H), 1.89 m (1H, C_2-H), 2.88 dd (1H, J_1 = 14.4 Hz, J_2 = 6.6 Hz, C_1-H), 3.05 dd (1H, J_1 = 14.4 Hz, J_2 = 3.6 Hz, C_1-H), 7.50–7.87 m (5H, Ph), Mass spectrum (*m/z*): 240 (M^+), 99, 98 (M^+-PhSO_2, M^+-PhSO_2H).

24-Ethyl-3α,5-cyclo-5α-cholest-22E-en-6-one (9). At — 78°C, a solution of 2.03 g of the sulfone (**6**) in 20 ml of THF was treated with 6 ml of 1.6 N butyllithium in hexane. After 25 min, a solution of 3.150 g of the aldehyde (**7**) in 20 ml of THF was added, and the reaction mixture was kept for 2 h. Then 7 ml of acetic anhydride was added and the resulting

mixture was left overnight at room temperature. It was evaporated, and the residue was dissolved in a mixture of 75 ml of ethyl acetate and 100 ml of methanol; this solution was cooled to −20°C and, with continuous stirring, 40 g of 6% sodium amalgam was added. After 6 h, 25 ml of water and 75 ml of AcOH were also added, and the resulting mixture was left overnight. Then it was evaporated, and the residue was diluted with water and extracted with chloroform. The extract was washed with saturated sodium bicarbonate solution, filtered through a layer of alumina, and evaporated, and the residue was chromatographed on a column of silica gel. Hexane−ether (10:1) eluted 2.388 g of compound (**9**) (68.9% yield). IR spectrum (cm^{-1}): 1695 (C=O). PMR spectrum (360 MHz, δ, ppm): 0.72 s (3H, 18-Me), 0.77−0.81 m (6H, 29-Me, 26-Me or 27-Me), 0.84 d (3H, J = 6 Hz, 27-Me or 26-Me), 1.00 s (3H, 19-Me), 1.02 d (3H, J = 6.6 Hz, 21-Me), 2.41 d (1H, J = 12 Hz, C_7−H), 4.96−5.16 m (2H, C_{22}−H, C_{23}−H). Mass spectrum (*m/z*): 410 (M^+), 395 (M^+−Me), 381 (M^+−Et), 299, 298, 297 (C_{20}−C_{22} cleavage).

Hydroxylation of Compound (9) with Osmium Tetroxide. A solution of 2.37 g of steroid (**9**) in 30 ml of pyridine was added to a solution of 1.47 g of osmium tetroxide in 20 ml of pyridine, and the mixture was kept for 24 h. After this, it was treated with a solution of 6 g of Na_2SO_3 and 1 ml of conc. H_2SO_4 in 60 ml of water at 55-60°C for 50 min and was then was diluted with water and extracted with chloroform. The extract was washed with water and evaporated, and the residue was chromatographed on a column of silica gel with elution by ether−hexane (1:2). The total yield was 87%. The following were isolated:

Fraction 1 — 860 mg of **(22S,23S,24S)-24-ethyl-22,23-dihydroxy-3α,5-cyclo-5α-cholestan-6-one (12)**. IR spectrum (cm^{-1}): 3420 (OH), 1690 (C=O). PMR spectrum (360 MHz, δ, ppm): 0.75 s (3H, 18-Me), 0.88 d (3H, J = 7.2 Hz, 27-Me or 26-Me), 0.94 d (3H, J = 7.2 Hz, 27-Me or 26-Me), 0.96 t (3H, J = 7.2 Hz, 29-Me), 1.00 s (3H, 19-Me), 1.04 d, (3H, J = 7 Hz, 21-Me), 2.08 m (1H, C_7−H), 2.42 d (1H, J = 12 Hz, C_7−H), 3.56−3.62 m (2H, C_{22}−H, C_{23}−H). Mass spectrum (*m/z*): 444 (M^+), 429 (M^+−Me), 426 (M^+−H_2O), 411 (M^+−Me−H_2O);

Fraction 2 — 704 mg of **(22S,23S,24R)-24-ethyl-22,23-dihydroxy-3α,5-cyclo-5α-cholestan-6-one (11)**. IR spectrum (cm^{-1}): 3430 (OH), 1690 C=O). PMR spectrum (360 MHz, δ, ppm): 0.74 s (3H, 18-Me), 0.93 d (3H, J = 7.2 Hz, 27-Me or 26-Me), 0.96 d (3H, J = 7.2 Hz, 27-Me or 26-Me), 0.967 t (H, J = 7.2 Hz, 29-Me), 1.00 s (3H, 19-Me), 1.02 d (3H, J = 7 Hz, 21-Me) 2.06 dd (1H, J_1 = 12 Hz, J_2 = 3.6 Hz, C_7−H), 2.42 d (1H, J = 12 Hz, C_7−Hβ), 3.56-3.77 m (2H, C_{22}−H, C_{23}−H). Mass spectrum (*m/z*): 444 (M^+), 415 (M^+−Et), 408 (M^+−$2H_2O$);

Fraction 3 — 612 mg of **(22R,23R,24R)-24-ethyl-22,23-dihydroxy-3α,5-cyclo-5α-cholestan-6-one (10)**. IR spectrum (cm^{-1}): 3360 (OH), 1695 (C=O). PMR spectrum (360 MHz, δ, ppm): 0.73 s (3H, 18-Me), 0.91-0.96 m (9H, 27-Me, 26-Me, 29-Me), 0.97 d (3H, J = 7.2 Hz, 21-Me), 1.02 s (3H, 19-Me), 2.04 m (1H, C_7−H), 2.42 d (1H, J = 12 Hz, C_7−H), 3.65-3.75 m (2H, C_{22}−H, C_{23}−H). Mass spectrum (*m/z*): 444 (M^+), 429 (M^+−Me), 426 (M^+−H_2O), 415 (M^+−Et), 408 (M−$2H_2O$).

(22R,23R,24R)-24-ethyl-22,23-dihydroxy-5α-cholest-2-en-6-one (13). A solution of 563 mg of compound (**10**) in 15 ml of dimethylformamide was treated with 600 mg of pyridine hydrobromide. The reaction mixture was boiled for 2 h, evaporated, and chromatographed on a column of silica gel with elution by ether−hexane (2:1). The resulting yield of compound (**13**) was 453 mg (80%). mp 140-142°C (ethanol). IR spectrum (cm^{-1}) 3440 (OH), 1710 (C=0). PMR spectrum (360 MHz, δ, ppm): 0.69 s (3H, 18-Me), 0.72 s (3H, 19-Me), 0.93 d (6H, J = 7.2 Hz, 21-Me, 26- or 27-Me), 0.95 d (3H, J = 7.2 Hz, 26- or 27-Me), 0.957 t (3H, J = 7.2 Hz, 29-Me), 2.35 d (1H, J = 3.6 Hz, C_7−Hβ or C_7−Hα), 2.39 d (1H, J = 3.6 Hz, C_7−Hβ or C_7−Hα), 3.70 m (2H, C_{22}−H, C_{23}−H), 5.58 m (1H, C_2− or C_3−H), 5.70 m (1H, C_2− or C_3−H). Mass spectrum (*m/z*): 444 (M^+), 429 (M^+−Me), 426 (M^+−H_2O), 360, 359 (C_{22}−C_{23} cleavage).

(22R,23R,24R)-24-ethyl-2α,3α,22,23-tetrahydroxy-5α-cholestan-6-one (14). With continuous stirring, a solution of 160 mg of steroid (**13**) in 5 ml of Py was added to a solution of 91 mg of osmium tetroxide in 2 ml of pyridine. The reaction mixture was kept at room temperature for 20 min, and then a solution of 0.8 g of Na_2SO_4 and 0.2 ml of concentrated sulfuric acid in 6 ml of water was added over 0.5 h, after which it was diluted with water and extracted with chloroform. The extract was evaporated, and the residue was chromatographed on a column of silica gel, giving 170 mg (98% yield) of compound (**14**). mp 247-249°C (ethanol). IR spectrum (cm^{-1}): 3445 (OH), 1710 (C=O). PMR spectrum (360 MHz, δ, ppm): 0.66 s (3H, 18-Me), 0.76 s (3H, 19-Me), 0.90-1.01 m (12H, 21-, 26;, 29-Me), 2.33 dd (1H, J_1 = 13.2 Hz, J_2 = 4.8 Hz, C_7−Hα), 2.73 dd (1H, J_1 = 12 Hz, J_2 = 3.6 Hz, C_5−H_α, 3.68-3.87 m (3H, C_{22}−H, C_{23}−H, C_2−Hα), 4.12 d (1H, J = 2.4 Hz, C_3−Hβ). Mass spectrum (*m/z*): 460 (M^+), 460 (M^+−H_2O), 445 (M^+−H_2O−Me), 427 (M−$2H_2O$−Me).

(22R,23R,24R)-24-Ethyl-2α,3α,22,23-tetraacetoxy-5α-cholestan-6-one (15). A solution of 110 mg of compound (**15**) in 3 ml of pyridine was treated with 1.5 ml of acetic anhydride, and the mixture was kept at 60°C for 24 h. Then it was evaporated, and the residue was chromatographed on a column of silica gel with elution by hexane−ether (1:1). The yield of

the tetraacetate (**15**) was 108 mg (89%). IR spectrum (cm^{-1}): 1740 (Ac), 1710 (C_6=O). PMR spectrum (360 MHz, δ, ppm): 0.68 s (3H, 18-Me), 0.82 s (3H, 19-Me), 0.90 d (3H, J = 7.2 Hz, 26- or 27-Me), 0.94 d (3H, J = 7.2 Hz, 26- or 27-Me), 0.95 t (3H, J = 4.8 Hz, 29-Me), 1.00 d (3H, J = 6.6 Hz, 21-Me), 1.98 s (6H, AcO), 2.01, 2.07 s (3H, AcO), 2.30 dd (1H, J_1 = 13.2 Hz, J_2 = 4.2 Hz, C_7−Hα), 2.54 dd (1H, J = 12 Hz, J = 4.8 Hz, C_5−Hα), 4.9 ddd (1H, J_1 = 10.8 Hz, J_2 = 4.8 Hz, J_3 = 2.4 Hz, C_2−Hα), 5.25 m (2H, C_{22}−H, C_{23}−H), 5.32 d (1H, J = 2.4 Hz, C_3−Hβ). Mass spectrum (*m/z*): 586 (M^+−AcOH), 526 (M^+−2AcOH).

(22R,23R,24R)-24-ethyl-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-cholestan-6-one (16). At −5 to 0°C, 0.3 ml of 30% hydrogen peroxide was added to a solution of 3 ml of trifluoroacetic acid in 7 ml of chloroform, and the mixture was kept at the same temperature for 20 min. Then a solution of 120 mg of the acetate (**15**) in 5 ml of chloroform was added, and the reaction mixture was kept at room temperature for 5 h. It was evaporated, and the residue was chromatographed on a column of silica gel with elution by hexane−chloroform (1:1). After this, a solution of 90 mg of the lactone so obtained in 9 ml of a 5% solution of KOH in methanol was boiled for 1 h.

The reaction mixture was cooled to room temperature and, after the addition of 10 ml of 25% hydrochloric acid, it was left for 1.5 h. It was then diluted with a saturated solution of sodium bicarbonate and extracted with chloroform. The yield of compound (**16**) was 35 mg (42.5%). mp 268-270°C. IR spectrum (cm^{-1}): 3420 (OH), 1715 (lactone). PMR spectrum (360 MHz, δ, ppm): 0.69 s (3H, 18-Me), 0.88-0.97 m (15H, 26-, 27-, 29-, 21-, 19-Me), 3.10 dd (1H, J_1 = 12 Hz, J_2 = 4.2 Hz, C_5−H), 3.61-3.72 m (3H, $W_{1/2}$ 22.8 Hz, C_{22}−H, C_{23}−H, C_2−Hα), 3.98 m (1H, $W_{1/2}$ 8.4 Hz, C_3−Hβ), 4.05 m (2H, $W_{1/2}$ 10.8 Hz, C_7−H). Mass spectrum (*m/z*): 458 (M^+−$2H_2O$H), 440 (M^+−3AcOH).

REFERENCES

1. L. Fieser and M. Fieser, Steroids, Reinhold (1959).
2. V. A. Khripach, F. A. Lakhvich, and V. N. Zhabinskii, Brassinosteroids [in Russian], Nauka Tekhnika, Minsk (1993).
3. F. A. Lakhvich, V. A. Khripach, and V. N. Zhabinskii, Vestsi Akad. Nauk BSSR, Ser. Khim. Navuk, No. 3, 99 (1990).
4. S. Takatsuto, N. Yazawa, N. Ikekawa, T. Morishita, and H. Abe, Phytochemistry, **22**, 1393 (1983).
5. V. A. Khripach, V. N. Zhabinskii, V. K. Ol'khovik, and A. A. Akhrem, Vestsi Akad. Navuk BSSR, Ser. Khim. Nauk, No. 6, 69 (1990).
6. V. A. Khripach, V. N. Zhabinskii, V. K. Ol'khovich, and F. A. Lakhvich, Zh. Org. Khim., **26**, 2200 (1990).
7. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, V. N. Zhabinskii, et al., USSR Inventors' Certificate 1,577,107 (1987).