The main source of formation of the peak of the $(M - 59)^+$ ion (the second most intense), is also the acetoxy group at C-13 (see scheme). Nevertheless, $(M - 59)^+$ ions with different compositions can be obtained by the elimination of the fragments $CHO + CH_2O$ and $OH + CH_2CO$ (see Fig. 1 and Table 3).

The $(M - 43)^+$ and $(M - 59)^+$ fragments of compound (V) partially decompose, splitting out the constituent OR_1 in the form of a molecule of the acid which leads to the appearance of ions with m/z 326 and 310, respectively (see Fig. 1; Tables and 3).

Thus, the main directions of the fragmentation of bases (I-V) are determined by the presence of the OH group at C-14 and, in contrast to the hetisine alkaloids of other groups [1], the elimination of the elements of ring A, B, and C with the formation of nitrogen-free fragments is uncharacteristic. Base (V) is characterized by a high selectivity of its breakdown due to the acetoxy group at C-13.

EXPERIMENTAL

MKh 1310 mass spectrometer with double focusing, SVP 5 system for the direct introduction of the sample, temperature of the ionization chamber 140-170°C, temperature of the heating ampul 100-160°C, ionizing voltage 70 V, collector current 16 μA. For the conditions for obtaining the MD spectra, see [4], and for the B/E = const spectra, see [5].

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A NEW SYNTHESIS OF BRASSICASTEROL

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UDC 547.92

A new method has been developed for obtaining brassicasterol – the initial compound for the synthesis of the natural brassinosteroid epibrassinolide.

In 1988, Ikekawa et al. [1] isolated from broad beans Vicia faba L. the new phytohormone 24-epibrassinolide (EB) (I) belonging to the class of brassinosteroids [2]. Its synthesis as one of the closest structural analogues of brassinolide [2] has been performed previously by American authors [3] and it was later shown that with respect to its level of plant growth stimulating activity it was comparable with brassinolide. This fact, and also the relatively wide distribution in nature of Δ^{22}-sterols with a carbon skeleton corresponding to the structure of (I) [ergosterol (III), brassicasterol (IV)], which permits them to be considered as convenient starting compounds for synthesis, attracted the intense attention of researchers to EB. A subsequent comparative study of various brassinosteroids under field conditions from the point of view of their influence on crop yield showed the advantageous nature of EB and good prospects of its practical use in agriculture [5, 6].

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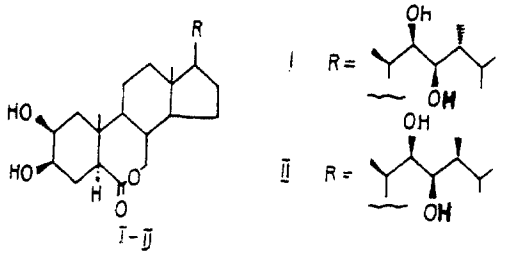
Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 90-93, January-February, 1992. Original article submitted April 25, 1991.

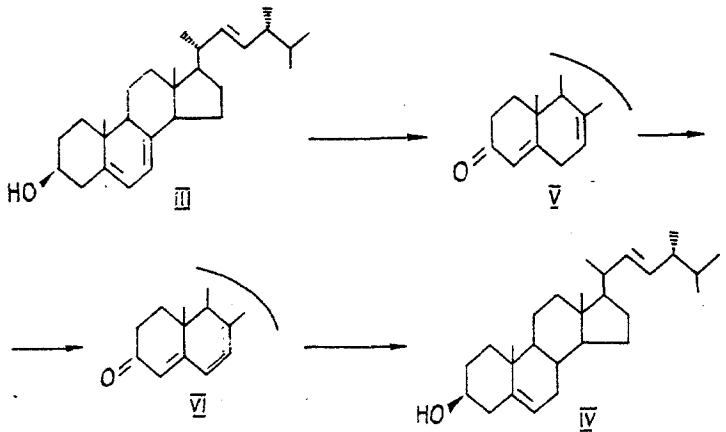
0009-3130/92/2801-0074$12.50



Since the amount of EB in natural materials is extremely low (less than 10^{-6}%), chemical synthesis may be regarded as the only source for satisfying demands for it. Among the syntheses of EB described at the present time, the most successful are based on the use of brassicasterol (IV) as the starting material or a key intermediate. The known method for obtaining brassicasterol by isolation from plant raw material [7] is of limited value, since the sterol obtained always contains, as impurities with similar structures, poriferasterol, stigmasterol, and a number of other compounds the level of which frequently exceeds that of brassicasterol. In view of this, at the present time the main synthetic methods for obtaining brassicasterol are those for which the starting compound is ergosterol.

Although it is fairly convenient, the direct method for converting ergosterol into brassicasterol that we have described previously [8, 9] nevertheless leads to a by-product – 5-dihydroergosterol, and the separation of the mixture is extremely difficult. Similar disadvantages and relatively low yields of desired product are also characteristic of other methods connected with the reduction of ergosterol [10] or its derivatives [11].

The aim of the present work consisted in the creation of a new and more effective method of synthesizing brassicasterol which permits the avoidance of the formation of the by-product 5-dihydroergosterol. For this purpose we studied a direct method of reduction including the initial transformation of ergosterol into the trienoketone (VI). Thus, the Oppenhauer oxidation of (III) with cyclohexanone in toluene in the presence of aluminum isopropanolate took place smoothly, giving a 90% yield of the trienoketone (V). The IR spectrum of (IV) contained the absorption band of a keto group at 1680 cm^{-1} and of a double bond conjugated with it at 1625 cm^{-1}. The PMR spectrum contained the signal of a vinyl proton in the α-position to the carbonyl at C-4, with δ 5.71 ppm. When an ethanolic solution of compound (V) was boiled in the presence of hydrochloric acid, the double bond in ring B underwent isomerization from the Δ^7 position to the Δ^6 position with the formation of compound (VI) having a dienoketone system. The IR spectrum of the 4,6-dieno-3-ketone (VI) differed from that of (V) by a lowering of the absorption frequency of the carbonyl group to 1670 cm^{-1} and that of the double bond to 1620 cm^{-1}, while in the PMR spectrum a weak-field multiplet (δ 6.11 ppm) corresponding to the resonance absorption of 6- and 7-vinyl protons included in a system of conjugation appeared. The reduction of the dienoketone (VI) with lithium in a mixture of tetrahydrofuran and liquid ammonia gave 74% of brassicasterol (IV) the structure of which agreed with the results of spectral methods.



The method of obtaining brassicasterol that has been considered includes three stages and leads to the desired product with a yield of 55%, which, in comparison with the method of [12], for example, is equivalent to a shortening of the process by three stages and to

an increase in yield by 30%. Thus, the proposed route to the production of brassicasterol is fairly effective and can be made the basis of the synthesis of 24R-brassinosteroids from ergosterol.

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 a WM-360 NMR spectrometer with a working frequency of 360 MHz in 5% $CDCl_3$ solutions with TMS as internal standard. Mass spectrometric characteristics were obtained on a Varian MAT-311 instrument at an energy of the ionizing ration of 70 eV. For the analytical modeling of the course of the reaction we used type LSL_{254} silica gel and Silufol UV-254 plates.

24R-Methylcholest-4,7,22-trien-3-one (V). A solution* of 4.0 g of ergosterol was boiled over calcium hydride in a Soxhlet extractor for 1 h to eliminate water completely. To the boiling solution was added 0.52 g of aluminum isopropanolate and 10 ml of dry cyclohexanone. The reaction mixture was boiled for 30 min and was then cooled to 80°C, and 1.3 ml of acetic acid and 0.6 ml of water were added. The resulting solution was cooled to 20°C, filtered through a layer of alumina, and separated. The residue was chromatographed on a column of silica gel with elution by hexane–ether (10:1). This gave 3.53 g (90%) of compound (V), mp 128-134°C (acetone). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1680 (C=O), 1625 (C=C). PMR spectrum: 0.60 (3H, s, 18-Me), 0.81 (3H, d, 26-CH_3, J = 6), 0.84 (3H, d, 27-CH_3, J = 6), 0.91 (3H, d, 21-Me, J = 7.2), 1.04 (3H, d, 28-CH_3, J = 7.2), 1.17 (3H, s, 19-Me), 5.18 (3H, m, 7-CH, 22-CH, 23-CH), 5.71 (1H, d, 4-CH, J = 0.3). Mass spectrum, m/z: 394 (M^+), 379 ($M^+ - CH_3$), 351 ($M^+ - C_3H_7$).

24R-Methycholest-4,6,22-trien-3-one (VI). A solution of 1 g of (V) in 100 ml of methanol was treated with 10 ml of hydrochloric acid, and the reaction mixture was boiled for 1.5 h. The solution was cooled to 30°C, partially evaporated, diluted with water, and extracted with chloroform. The organic phase was washed with sodium bicarbonate solution and was dried over sodium sulfate. The product was crystallized from acetone. This gave 0.82 g (82%) of (VI), mp-106-108°C (acetone). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1666 (C=O), 1620 (C=C). PMR spectrum: 0.77 (3H, s, 18-CH_3), 0.82 (3H, d, 26-CH_3, J = 6), 0.84 (3H, d, 27-CH_3, J = 6), 0.92 (3H, d, 21-CH_3, J = 7.2), 1.03 (3H, d, 28-CH_3, J = 7.2), 1.16 (3H, s, 19-CH_3), 5.18 (2H, m, 22-CH, 23-CH), 5.68 (1H, s, 4-CH), 6.11 (2H, m, 6-CH, 7-CH). Mass spectrum, m/z: 394 (M^+), 379 ($M^+ - CH_3$), 351 ($M^+ - C_3H_7$).

3β-Hydroxy-24R-methylcholest-5-ene (III). A solution of 1.565 g of (VI) in 45 ml of tetrahydrofuran was added with stirring to a mixture of 230 ml of liquid ammonia and 100 ml of tetrahydrofuran. Then 1.4 g of ammonium chloride and 0.25 g of lithium were added. As the reaction proceeded, additional amounts of 1.4 g of ammonium chloride and 0.3 g of lithium were added twice. After 5 h, the mixture was diluted with water and extracted with chloroform. The extract was dried over sodium sulfate and evaporated, and the residue was chromatographed on a column of silica gel with elution by hexane–ether (5:1). This gave 1.173 g (74%) of (III), mp 135-140°C (acetone). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3440 (OH). PMR spectrum: 0.69 (3H, s, 18-CH_3), 0.82 (3H, d, 26-CH_3, J = 6), 0.84 (3H, d, 27-CH_3, J = 6), 0.91 (3H, d, 21-CH_3, J = 7.2), 1.01 (3H, s, 19-Me), 1.04 (3H, d, 28-CH_3, J = 7.2), 1.04 (3H, d, 28-CH_3, J = 7.2), 3.51 (1H, m, H-3α), 5.19 (2H, m, 22-CH, 23-CH), 5.35 (1H, m, 6-CH). Mass spectrum, m/z: 398 (M^+), 380 ($M^+ - H_2O$), 367 ($M^+ - H_2O - CH_3$), 355 ($M^+ - C_3H_7$).

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*Solvent not specified – Translator

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MASS SPECTRA OF FUROSTANOL GLYCOSIDES

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UDC 543.51+547.918+547.914

The mass-spectrometric fragmentation of furostanol glycosides under electron impact is described, and a comparison is made of these spectra with the secondary-emission spectra obtained by the FABMS and LSIMS methods.

Electron-impact (EI) mass spectrometry has been used successfully in the investigation of glycosides of the spirostan series [1, 2] containing a small number of carbohydrate units. However, with an increase in this number the polarity of the unmodified glycosides also increases, which makes the EI mass spectra less informative. The efficacy of the use of this mass-spectral method in the study of furostanol glycosides becomes even lower because of their higher polarity, in comparison with that of the spiro analogues, the instability of the $M^{+\cdot}$ ions, and the less characteristic nature of the mass spectra. These factors have impelled a search for accessible highly sensitive methods of mass spectrometry with "mild" ionization capable of expanding the range of compounds of this class that can be studied and also of being used where it is impossible to obtain EI spectra without chemical modification of the compounds under investigation.

The methods of "mild" ionization that have been used successfully in recent years in the mass spectrometry of natural compounds include secondary-emission methods of ionization. Those that are used most frequently are the mass spectrometry of secondary ions from a liquid matrix (LSIMS) and fast-atom bombardment (FAB). In preceding papers on the mass spectrometry of steroid glycosides [3, 4] we have shown the applicability of the LSIMS method for the study of unmodified glycosides of the spirostan series. In the present communication we give the results of an investigation of the spectra of glycosides of the furostan series isolated from Nolina microcarpa – nolinofurosides A (I), C (II) [5], F (III) [6], G(IV), and H (V) [7] – and also the products of the desulfation of the last two glycosides – compounds (VI) and (VII) [7], obtained by the LSIMS and FAB methods. These spectra were also compared with the EI spectra of the above-mentioned compounds [with the exception of the glycosides (IV) and (V)], which have proved to be fairly informative (see scheme at top of following page).

ELECTRON-IMPACT MASS SPECTRA

The presence of sulfur groups in the steroid moieties of compounds (IV) and (V) led to their thermal decomposition in the inlet system and to the impossibility of obtaining EI

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0009-3130/92/2801-0077$12.50