Chemistry of Natural Compounds, Vol. 31, No. 5, 1995

SYNTHESIS OF THE 3,22,23-TRIACETATES OF 28-HOMOTYPHASTEROL AND ITS (22S,23S)- ISOMER

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UDC 547.92

Acetates of the brassinosteroids 28-homotyphasterol and (22S,23S)-28-homotyphasterol have been obtained from stigmasterol. The scheme of synthesis includes the epoxidation of the corresponding 2,22-diene-6-ketone, reduction of the 2α,3α:22,23-diepoxy-6-ketone formed with lithium tetrahydroaluminate to a 22,23-epoxy-3α,6β-diol, oxidation of the 6β-hydroxy group to a 6-ketone, and conversion of the 22,23-epoxides into cis-22,23-diols.

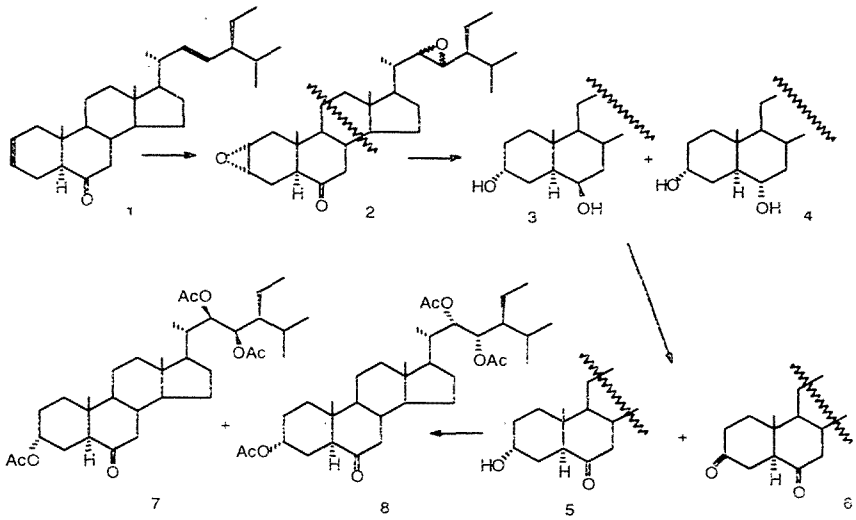
Progress in the chemistry and biochemistry of the plant growth regulators brassinosteroids is determined to a considerable degree by advances in the development of new effective schemes for their synthesis from available steroid raw material. At the present time, a fairly large number of diverse methods have been developed for synthesizing the basic brassinosteroids (brassinolide, 24-epibrassinolide, castasterone, brassinone, etc.), the molecules of which are characterized by the presence of 2α,3β- and (22R,23R)-22,23-diol groupings and a lactone or ketone function in ring (*B*) [1]. At the same time, far less attention has been devoted to the development of methods for obtaining 3α,22,23-trihydroxy-6-ketobrassinosteroids such as typhasterol, which has the structure of (22R,23R,24S)-3α,22,23-trihydroxy-5α-ergostan-6-one [2, 3]. The latter circumstance is probably connected with their appreciable lower biological activity than that of brassinolide and 24-epibrassinolide [3]. However, brassinosteroids like typhasterol are apparently intermediates in the biosynthesis of brassinolide and are therefore of undoubted interest for biochemical investigation.

We have previously [4] proposed a new scheme for synthesizing 3α-hydroxy-6-ketosteroids via 2α,3α-epoxy-6-ketones and have shown its applicability to the preparation of (22S,23S)-28-homotyphasterol. The object of the present investigation was the possibility of using this scheme for the synthesis of brassinosteroids containing, in addition to a 3α-hydroxy-6-keto grouping, the (22R,23R)-22,23-diol function that is characteristic for the natural hormones [5].

In following the selected scheme of synthesis, we obtained the 2,22-diene-6-ketone (**1**) from stigmasterol as a result of tosylation, solvolysis of the tosylate, oxidation of the 3α,5-cyclo-6-alcohol, opening of the three-membered ring in the resulting 3α-cyclo-6-ketone with hydrogen bromide, and then dehydrobromination of the 3β-bromo-6-ketone. Compound (**1**) contains, in addition to the 6-keto group, two double bonds suitable for the introduction of a 3α,22,23-triol grouping. As the result of an interaction of the dienone (**1**) with an excess of *m*-chloroperbenzoic acid in chloroform the 2α,3α:22,23-diepoxide (**2**) was obtained with a yield of about 70% in the form of a mixture of (22R,23R)- and (22S,23S)- isomers. The structure of (**2**) was shown unambiguously with the aid of NMR spectra. In the first place, the spectrum of steroid (**2**) lacked the signals of C_2-H, C_3-H, $C_{22}-H$, and $C_{23}-H$ protons, the presence of which was characteristic for the spectrum of the initial dienone (**1**). In the second place, the PMR spectrum of compound (**2**) contained the signals of four methine protons geminal to the epoxy groups, at 2.47, 2.72, 3.12, and 3.26 ppm. A comparison of the chemical shifts and forms of these signals in the spectrum of the diepoxide (**2**) with the analogous characteristics in the spectrum of the 2α,3α-monoepoxide obtained from the diene (**1**) [4] proved to be useful. From this it was possible to deduce that the multiplets at 3.12 and 3.26 ppm related to the resonance absorption of, respectively, the $C_2-H\beta$ and $C_3-H\beta$ methine protons geminal to the 2α,3α-epoxide ring. Thus, the remaining signals, at 2.47 and 2.72 ppm, the integral intensity of which corresponded to the absorption of two atoms, must be ascribed to the $C_{22}-H$ and $C_{23}-H$ atoms geminal to the 22,23-epoxide ring.

Institute of Bioorganic Chemistry, Belarus Academy of Sciences, 220141, Minsk, ul. Zhodinskaya, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 694-699, September-October, 1995. Original article submitted January 30, 1995.

 0009-3130/95/3105-0584$12.50



Because of steric hindrance, the 22,23-epoxy group in steroids of the stigmastane series is chemically inert, and does not undergo, for example, the action of lithium tetrahydroaluminate [7]. We therefore hoped that, starting from the diepoxyketone (**2**), it would be possible to obtain by the method of [4] the 3α-hydroxy-6-ketone in which the 22,23-epoxy group had remained unchanged. In actual fact, in agreement with expectations, it was established that the interaction of steroid (**2**) with lithium tetrahydroaluminate in anhydrous ether led to the formation of two substances (**3**) and (**4**), which were isolated by column chromatography with yields of 32 and 8%, respectively. Judging from the PMR spectra, steroids (**3**) and (**4**) each contained a 22,23-epoxy group. The IR spectra of the substances under discussion contained no absorption bands in the 1700 cm^{-1} region, and from this it was possible to deduce the absence of 6-keto groups from their structures. The presence at 3.76 and 4.17 ppm in the PMR spectrum of steroid (**3**) of the signals of methine protons geminal to secondary hydroxy groups showed its structure as a 3,6-diol. It followed unambiguously from the half-width of these signals ($W_{1/2}$ 7.5 Hz) that both hydroxy groups were axial. A comparison of the chemical shifts of the methine protons geminal to the hydroxy groups in diols (**3**) with the corresponding parameters in the PMR spectra of the 3α,6β-diols obtained previously [4] from 2α,3β-epoxy-6-ketones showed their practical coincidence.

Thus, the main product of the reduction of the 2α,3β:22,23-diepoxy-6-keto-steroid (**2**) must be ascribed the structure of the 22,23-epoxy-3α,6β-diol (**3**).

From the PMR spectrum it was possible to conclude that the minor compound (**4**) contained, in addition to a 22,23-epoxy function, a 3α-hydroxy group. To this structural fragment corresponded the presence in the spectrum of the signal of a methine proton geminal to it, C_3-H_β, with δ 4.14 ppm which, judging from its half-width ($W_{1/2}$ 7 Hz), had an equatorial orientation. The signal of the methine proton geminal to the second hydroxy groups in the spectrum had the form of a broadened multiplet at 3.38 ppm with a half-width $M_{1/2}$ of 25 Hz, which showed its axial orientation. From these facts it is possible to draw the conclusion that in compound (IV) the 6-hydroxy group had the equatorial orientation and it was a 3α,6α-diol.

When the 3α,6β-diol (**3**) was subjected to Jones oxidation with chromic acid in acetone, we obtained 32 and 42% yields of the 3α-hydroxy-6-ketone (**5**) and the 3,6-diketone (**6**). As followed from the PMR spectra, in both compounds, the 22,23-epoxy group had been retained. The IR spectra of steroids (**5**) and (**6**) contained the bands of stretching vibrations of carbonyl groups at 1700 and 1710 cm^{-1}, respectively. In the PMR spectrum of the ketoalcohol (**5**), only the signal of the methine proton geminal to the 3α-hydroxy group, C_3-H_β (δ 4.16 ppm, $W_{1/2}$, 8 Hz) was present. This unambiguously showed the structure of the substance under discussion. Furthermore, the presence in steroids (**5**) of a 3α-hydroxy-6-keto group caused a characteristic downfield shift of the signals of the C_5-H_α and C_7-H_β protons. In its turn, in the PMR spectrum of the second product of the oxidation of the 3α,6β-diol (**3**), the signals of methine protons geminal to hydroxy groups were completely absent, which confirmed its structure as the 3,6-diketone (**6**).

For the synthesis of *cis*-22,23-diols, from 22,23-epoxides a procedure has been proposed which includes their opening with hydrogen bromide, acetylation of the *trans*-bromohydrins formed, and nucleophilic replacement of the bromine atoms by acetoxy groups, taking place with reversal of the configuration [1]. Since all the reactions take place stereo-selectively, (22R,23R)-diols are, of course, obtained only from (22R,23R)-22,23-epoxysteroids. In their turn, the (22S,23S)-

22,23-epoxides are converted into (22S,23S)-22,23-diols. The application of this sequence to the 22,23-epoxysteroid (**5**) enabled the (22R,23R)-3α,22,23-triacetoxy-6-ketone (**7**) and its (22S,23S)- isomer (**8**) to be obtained with yields of 26 and 23%, respectively, after chromatographic separation of the reaction products.

The structures of the substances mentioned were shown with the aid of spectra. Thus, in the PMR spectrum of steroid (**7**) a downfield shift to 5.11 ppm of the signal of the C_3-H_β proton as compared with its position in the spectrum of the initial substance, caused by the acetylation of the 3α-hydroxy group, was observed. The spectrum lacked the signals of methine proton geminal to a 22,23-epoxide ring. At the same time, the signals of the $C_{22}-H$ and $C_{23}-H$ protons in the form of broad doublets at 5.18 and 5.32 ppm with splitting constants J = 9 Hz showed the presence of a 22,23-diacetoxy grouping in compound (**7**). Its (22R,23R)-configuration followed from a comparison of the chemical shifts and forms of splitting of the signals with the corresponding parameters in the PMR spectra of brassinosteroid acetates with the same stereochemical structure (see, for example, [8]). The structure of substance (**8**) was shown by an analysis of its PMR spectrum analogously.

In terms of chemical structure, substance (**8**) is 28-homotyphasterol 3,22,23-triacetate, the synthesis of which has been described previously [8]. A comparison of the results given in that paper with those that we have obtained show their approximately identical efficacy. It must be mentioned that 3α-hydroxy-22,23-epoxy-6-ketosteroids of type (**5**) that have become available as the result of our investigation are of interest as potential biologically active compounds.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were obtained on a UR-20 instrument. The PMR spectra of solutions in deuterochloroform were recorded on a Bruker AC-200 NMR spectrometer with a working frequency of 200 MHz. Chemical shifts are given relative to TMS as internal standard. The mass spectrometric results were obtained on a Varian MAT-311 instrument at an energy of the ionizing electrons of 70 eV.

(24S)-2α,3α:22,23-Diepoxy-5α-stigmastan-6-one (2). A solution of 4.16 g of the dienone (1) (obtained from stigmasterol by the method of [4]) in 89 ml of chloroform was treated with 4.52 g of *m*-chloroperbenzoic acid, and the mixture was left at room temperature for 23 h. Then 60 ml of saturated sodium bicarbonate solution was added and stirring was carried out with a magnetic stirrer for 35 min. The organic layer was separated from the aqueous layer and washed with water, and the solvent was evaporated in vacuum. The residue was deposited on a column of silica gel and was chromatographed with elution by hexane–ether (1:1). This gave 3.04 g of the diepoxide (**2**) in the form of a mixture of the (22R,23R)- and (22S,23S)- isomers. Yield 68%, mp 114-118°C.

IR spectrum (KBr, ν, cm^{-1}): 1710 (C=O). PMR spectrum (δ, ppm): 0.65 and 0.67 (s, 18-Me), 0.70 (s, 19-Me), (0.92 d, J = 7 Hz, 26-Me), 0.94 (d, J = 7 Hz, 27-Me), 0.96 (t, J = 7 Hz, 29-Me), 1.04 (d, J = 5 Hz, 21-Me), 2.47 m, 2.72 (dd, J_1 = 3 Hz, J_2 = 7 Hz, 2H, $C_{22}/C_{23}-H$), 3.12 (m, $W_{1/2}$ = 11.5 Hz, C_2-H_β), 3.26 (m, $W_{1/2}$ = 8 Hz, C_3-H_6).

Reduction of the Diepoxyketone (2). At room temperature, with continuous stirring, 4.0 g of lithium tetrahydroaluminate was added in portions to a solution of 5.88 g of the steroid (**2**) in 170 ml of absolute ether. The reaction mixture was stirred with a magnetic stirrer for 10 min, treated with ethyl acetate, and deposited on a column of alumina. Chromatography with elution by hexane–ether (5:1) and then (1:1) and ether gave:

(24S)-22,23-Epoxy-5α-stigmastane-3α,6β-diol (3). 1.88 g. Yield 32%, mp 166-169°C (hexane–acetone).

IR spectrum (KBr, ν, cm^{-1}): 3400 (OH). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.94 (d, J = 7 Hz, 26-Me), 0.96 (d, J = 7.5 Hz, 27-Me), 0.98 (t, J = 7.5 Hz, 29-Me), 1.02 (s, 19-Me), 1.04 (d, J = 5 Hz, 21-Me), 2.50 m, 2.76 (dd, J_1 = 3 Hz, J_2 = 7 Hz, 2H, $C_{22}/C_{23}-H$), 3.76 (m, $W_{1/2}$ = 7.5 Hz, C_6-H_α), 4.18 (m, $W_{1/2}$ = 7.5 Hz, C_3-H_β).

(24S)-22,23-Epoxy-5α-stigmastane-3α,6α-diol (4). 0.48 g. Yield 8%, mp 199-204°C (ether–ethyl acetate).

IR spectrum (KBr, ν, cm^{-1}): 3330 (OH). PMR spectrum (δ, ppm): 0.66 (s, 18-Me), 0.79 (s, 19-Me), 0.93 (d, J = 7 Hz, 26/27-Me), 0.98 (t, J = 8 Hz, 29-Me), 1.03 (d, J = 5 Hz, 21-Me), 2.50 m, 2.76 (dd, J_1 = 3 Hz, J_2 = 7 Hz, 2H, $C_{22}/C_{22}-H$), 3.38 (m, $W_{1/2}$ = 25 Hz, C_6-H_β), 4.14 (m, $W_{1/2}$, 7 Hz, C_3-H_β).

(24S)-3α-Hydroxy-22,23-epoxy-5α-stigmastan-6-one (5) and (24S)-22,23-Epoxy-5α-stigmastane-3,6-dione (6). With stirring by a magnetic stirrer, 1.2 g of steroid (**3**) was dissolved in 125 ml of acetone. Then the flask was placed in an ice bath, and 4 ml of 8 N chromic acid was added gradually. The reaction mixture was kept for 20 min and was then poured into water and extracted with chloroform. The extract was washed with water, and the solvent was evaporated off in vacuum. The residue was deposited on a column of silica gel and chromatographed with elution by chloroform. This gave:

The Diketone (5). 0.503 g. Yield 42%, mp 181-185°C (ether–ethyl acetate). IR spectrum (KBr, ν, cm^{-1}): 1710 (C=O). PMR spectrum (δ, ppm): 0.69 (s, 18-Me), 0.96 (s, 19-Me), 0.93 (t, J = 3.5 Hz, 29-Me), 0.94 (d, J = 7 Hz, 26/27-Me), 1.02 (d, J = 7 Hz, 21-Me), 2.75 (dd, J_1 = 2 Hz, J_2 = 5 Hz, C_5-H_α).

The Ketoalcohol (6). 0.380 g. Yield 32%, mp 155-160°C (ether–ethyl acetate).

IR spectrum (KBr, ν, cm^{-1}): 3380 (OH), 1700 (C=O). PMR spectrum (δ, ppm): 0.67 (s, 18-Me), 0.74 (s, 19-Me), 0.94 (d, J = 7 Hz, 26/27-Me), 0.97 (t, J = 7 Hz, 29-Me), 1.04 (d, J = 5 Hz, 21-Me), 2.31 (dd, J_1 = 4.5 Hz, J_2 = 13 Hz, C_7-H_β), 2.50 (dd, J_1 = 2.5 Hz, J_2 = 7 Hz), 2.68-2.80 m (3H, C_5-H_α, $C_{22}/C_{23}-H$), 4.16 (m, $W_{1/2}$ = 8 Hz, C_3-H_β).

28-Homotyphasterol 3,22,23-Triacetate (7) and (22S,23S)-28-Homotyphasterol 3,22,23-Triacetate (8). With stirring by a magnetic stirrer, 10 ml of acetic acid and 5.5 ml of hydrobromic acid were added in portions to a solution of 0.5 g of epoxide (7) in 6 ml of chloroform. The reaction mixture was stirred for 3 h and was then poured into water and extracted with chloroform. The organic layer was washed successively with saturated sodium bicarbonate solution and water and the solvent was evaporated off in vacuum. The residue (0.5 g) was dissolved in 10 ml of pyridine, and to this solution was added 0.1 g of dimethylaminopyridine and 2 ml of acetic anhydride, and the mixture was kept at room temperature for 2 days. Then the solvent was evaporated off, the residue was dissolved in chloroform, and the solution was washed successively with 10% hydrochloric acid, saturated sodium bicarbonate solution, and water. After elimination of the solvent, 50 ml of acetic acid and 3 g of potassium acetate were added and the mixture was boiled under reflux for 17 h. Then it was cooled to room temperature and the solvent was evaporated off. The residue was dissolved in chloroform and the solution was poured into water. The separated organic layer was washed with saturated sodium bicarbonate solution and with water and was passed through a layer of alumina. The filtrate was evaporated in vacuum and the residue was deposited on a column of silica gel and chromatographed, with elution by petroleum ether–chloroform (1:1). The resulting mixture of substances (0.34 g) was dissolved in 7 ml of pyridine, and, after the addition of 0.1 g of dimethylaminopyridine and 2 ml of acetic anhydride, was left at room temperature for two days. The solvent was evaporated off in vacuum, the residue was dissolved in chloroform, the chloroform solution was diluted with water, and the organic layer was separated off and was washed successively with water, 10% hydrochloric acid, sodium bicarbonate solution, and water. After elimination of the solvent, 15 ml of acetic acid and 1.5 g of potassium acetate were added and the mixture was boiled under reflux for 14 h. Then it was cooled to room temperature and was filtered through a layer of alumina. The solvent was evaporated off in vacuum, the residue was dissolved in chloroform, and the solution was poured into water and extracted with chloroform. The extract was treated with water and sodium bicarbonate solution. The solvent was evaporated off in vacuum and the residue was deposited on a column of alumina and was chromatographed with elution by petroleum ether–chloroform (2:1). This gave:

The Triacetate (8). 0.154 g. Yield 23%, mp 173-176°C (ethanol).

IR spectrum (KBr, ν, cm^{-1}): 1740, 1245 (AcO), 1715 (C=O). PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.75 (s, 19-Me), 0.80 (d, J = 6 Hz, 26/27-Me), 0.85 (t, J = 6 Hz, 29-Me), 0.95 (d, J = 7 Hz, 21–Me), 2.06 (s, 3 × AcO), 2.55 (m, $W_{1/2}$ = 16 Hz, C_5-H_α), 5.00-5.20 (3H, C_3-H_β and $C_{22}/C_{23}-H$). Mass spectrum, *m/z*: 528 (M^+-AcOH).

The Triacetate (7). 0.173 g. Yield 26%, mp 112-115°C (ether).

IR spectrum (KBr, ν, cm^{-1}): 3440 (OH), 1745, 1250 (AcO), 1710 (C=O).

PMR spectrum (δ, ppm): 0.70 (s, 18-Me), 0.75 (s, 19-Me), 0.84 (d, J = 7 Hz, 21-Me), 0.96 (d, J = 7 Hz, 26-Me), 0.98 (t, J = 7 Hz, 29-Me), 1.04 (d, J = 7 Hz, 27-Me), 1.98 (s, AcO), 2.04 (s, AcO), 2.06 (s, AcO), 2.32 (dd, J_1 = 4 Hz, J_2 = 13 Hz, C_7-H_β), 2.57 (quintet, J = 8 Hz, C_5-H_α), 5.11 (m, $W_{1/2}$ = 8 Hz, C_3-H_β), 5.18 (d, J = 9 Hz, $C_{22}-H$), 5.32 (d, J = 9 Hz, $C_{23}-H$). Mass spectrum, *m/z*: 588 (M^+).

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