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SYNTHESIS OF 7-OXO- AND 7-HYDROXY- DERIVATIVES OF STIGMASTEROL

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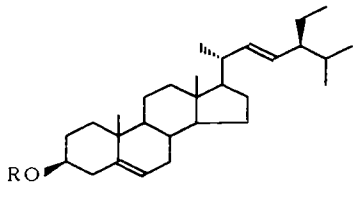
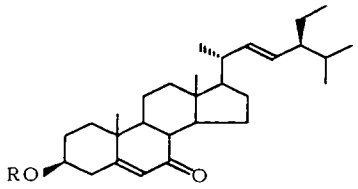
The phytosteroids 3β-hydroxy-(24S)-stigmast-5,22E-dien-7-one and (24S)-stigmasta-5,22E-diene-3β,7β-diol have been synthesized from stigmasterol.

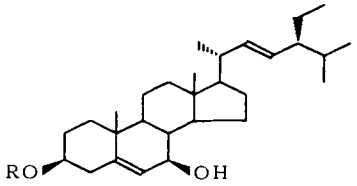
Stigmasterol (**1a**) is one of the main steroids of plants. Together with β-sitosterol and campesterol, this substance ensures the functioning of plant cell membranes. Moreover, the biosynthesis of various biologically active steroids necessary for the normal vital activity of plants starts from phytosterols, including stigmasterol. Compounds of this type include, for example, the hydroxysteroids (**3a-4a**) detected in plants [1, 2], the structures of which are characterized by the same carbon skeleton as in the stigmasterol molecule. It has been established by numerous investigations [4-9] that phytosterol derivatives with oxygen-containing functions at C-7, including steroids (**2a-4a**), possess a pronounced antitumoral activity. This circumstance has induced us to undertake the synthesis of these substances from the commercially available stigmasterol, which is used as a raw material in drug manufacture. In the present paper we give the results of this investigation, which is a continuation of syntheses of 6-oxophytosteroids from β-sitosterol [10] and stigmasterol [11] that we have performed previously.



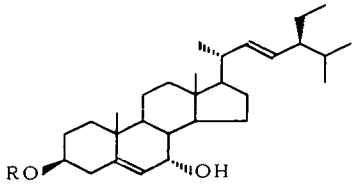
1. a-b



2. a-b



3. a-b



4. a-b

a) R=H; b) R=Ac

In the stigmasterol molecule, the H-7 atom is in an allyl position in relation to the 5(6) double bond, and, for this reason, it is activated. Although precisely the same situation exists for the H-4 atom, the selective oxidation of 5(6)-unsaturated steroids at C-7 is possible, for example, with the complex of chromium trioxide and pyridine, which has the structure of pyridinium dichromate [12]. Since, under these conditions, the 3β-hydroxy group in the stigmasterol molecule would also be oxidized, it was necessary to protect it. For this purpose, we first converted stigmasterol by acetylation with acetic anhydride in pyridine into the known acetate (**1b**) in quantitative yield.

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The activity of pyridinium dichromate falls considerably on storage. For the allyl oxidation of stigmasterol acetate we therefore used a method providing for the preparation of this reagent immediately before the reaction. It must be mentioned that in allyl oxidation using pyridinium dichromate it is very difficult to bring the reaction to completion. Usually, a certain amount of the initial compound remains, and attempts at the further transformation of this by the addition of more reagent or by increasing the time or raising the temperature of the reaction lead to a fall in the amount of required product as the result of further oxidation. In our case, when the reaction was performed in a mixture of pyridine and chloroform at room temperature the initial compound (**1b**) was also isolated, with a yield of about 50%.

At the same time, the expected reaction product (**2b**) was obtained with a yield of about 40%. Its structure was determined unambiguously from its UV, IR, and PMR spectra. For example, the UV spectrum of the substance under discussion contained the band at 236 nm that is characteristic for α,β-unsaturated ketones. In the IR spectrum, this structural fragment was responsible for the bands of stretching vibrations of the keto group at 1680 cm^{-1} and of the double bond conjugated with it at 1630 cm^{-1}. The fact that the oxidation product possessed the structure of a Δ^5-7-ketone, and not that of a Δ^5-4-ketone can well be seen from its PMR spectrum, in which the signal of the H-6 vinyl proton appeared at 5.70 ppm in the form of a doublet with a small splitting constant (J = 1.0 Hz) due to long-range spin-spin coupling.

The presence of a 3β-acetoxy group in the structure of compound (**2b**) was shown, in the first place, by a three-proton singlet at 2.05 ppm corresponding to its methyl moiety, and, in the second place, by the chemical shift and form of the signal of the H-3α proton geminal to it, which had the form of a broad multiplet at 4.72 ppm. Since the spectrum of compound (**2b**) contained signals of the H-22 and H-23 vinyl protons at 5.01 and 5.18 ppm, the presence of which is also characteristic for the spectrum of stigmasterol, it was posible to conclude with confidence that the 22(23)-double bond in it had been retained.

When the acetoxy group in steroid (**2b**) was hydrolyzed under the action of potassium carbonate in methanol, we obtained the desired hydroxyenone (**2a**) with a yield of 78%. The absence of an acetoxy group from compound (**2a**) was shown by the fact that its IR spectrum did not contain the bands at about 1740 and 1250 cm^{-1} that are characteristic for such a group. Furthermore, the PMR spectrum of the substance under discussion lacked a signal of the methyl protons of an acetoxy group in the region of about 2 ppm. An extremely characteristic feature was an upfield shift by 3.68 ppm of the signal of the H-3α methine proton, showing its position geminal not to an acetyl group but to a hydroxy group.

Of importance for proving the structure of phytosteroid (**2a**) was the presence of a band at 239 nm in its UV spectrum and of bands at 1670 and 1630 cm^{-1} in its IR spectrum, showing the presence of a Δ^5-7-keto grouping in its molecule. An analogous conclusion was provided by its PMR spectrum in which — as also in the spectrum of the acetate (**2b**) — there was a signal of the C-6 vinyl proton at 5.69 ppm. The retention of the 22(23)-double bond in compound (**2a**) could also be deduced easily from its PMR spectrum.

It is also possible to synthesize the 7-hydroxysterols (**3a**) and (**4a**) from the acetoxyenone (**2b**). For this purpose, we studied the reduction of this substance with sodium tetrahydroborate. When this reaction was performed at room temperature in a mixture of methanol and dioxane not only was the 7-oxo group reduced but the 3β-acetoxy group underwent partial hydrolysis. The 3-acetates (**3b** and **4b**) of the 3β,7β-diol and the 3β,7α-diol, with a predominance of the former, were isolated with a yield of 87% in the form of an inseparable mixture. The free 3β,7β-diol (**3a**) and 3β,7α-diol (**4a**) were isolated in small yield as minor products of this reaction.

Since we were unable to separate compounds (**3b**) and (**4b**), their structures were determined by analyzing a sample of their mixture. Extremely important for proving the structures of these compounds was the absence from their IR spectrum of an absorption band of α,β-unsaturated ketones at 1680 cm^{-1}. In the PMR spectrum, together with others, there were signals in the 3.8-3.9 ppm region due to the resonance absorption of the H-7α and H-7β protons geminal to 7β- and 7α-hydroxy groups, respectively. Hydrolysis of the 3β-acetoxy groups in compounds (**3b**) and (**4b**) under the action of potassium carbonate in methanol gave the 3β,7β-diol (**3a**) and the 3β,7α-diol (**4a**) as the main and minor reaction products, respectively.

It must be mentioned that, although the chromatographic characteristics of the free alcohols (**3a**) and (**4a**) differed considerably in comparison with those of the corresponding 3-acetates, nevertheless their isolation in pure form was fairly laborious and required several successive separations by column chromatography. The structures of these substances were reliably shown with the aid of their spectra. Thus, the IR spectra of diols (**3a**) and (**4a**) had broad absorption bands of OH groups at about 3500 cm^{-1} and lacked absorption bands corresponding to the stretching vibrations of acetoxy and oxo groups. In the PMR spectrum of the 3β,7β-diol, together with the signals of the H-22 and H-23 vinyl protons there was the signal of the H-6 vinyl proton in the form of a broadend singlet with δ 5.29 ppm. Such an upfield shift may indicate that in this compound there was no conjugation of a 5(6)-double bond with the 7-oxo group, as in compounds (**2a-b**) since the latter had been converted into a hydroxy group as a result of reduction.

In the spectrum at 3.55 ppm there was a multiplet of the H-3α proton geminal to the 3β-hydroxy group and having the axial orientation, to judge from the half-width of the signals (W/2 = 24 Hz). Moreover, a signal of the H-7α methine proton was seen at 3.85 ppm. Attention is attracted by the fact that this signal had the form of a doublet with a splitting constant J = 8 Hz due to vicinal interaction with the H-8β methine proton. Such a large value of the constant can correspond only to a mutual quasi-axial position of the protons. It follows from this that the H-7 atom had the quasi-axial (i.e., α-) orientation and, consequently, the 7-hydroxy group geminal to it had the β-orientation. This conclusion was confirmed by the absence of a spin-spin coupling constant between the H-6 and H-7 atoms. This is possible if the dihedral angle beween the atoms is about 90°, which is the case only if the 7-hydroxy group is β-oriented.

The structure of the 3β,7α-diol (**4a**) was shown analogously. Attention is attracted by the fact that the signal of the H-7 atom in the spectrum had the form of a complex multiplet with a half-width W/2 = 11 Hz. From its magnitude it was possible to conclude that this atom had the quasi-equatorial (i.e., β-) orientation. In addition, the β-orientation of H-7 and, consequently, the α-orientation of the OH group were confirmed by the existence between H-6 and H-7 of vicinal coupling with a constant J = 5.0 Hz, the value of which it was possible to determine from the multiplicity of the signal of the first proton.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra (tablets in KBr) were obtained on a UR-20 instrument. UV spectra were recorded on a Specord M-400 instrument, and PMR spectra on a Bruker AC-200 NMR spectrometer with a working frequency of 200 MHz. Chemical shifts are given relative to TMS as internal standard.

3β-Acetoxy-(24S)-stigmasta-5,22E-dien-7-one (2b). With stirring, 6.5 g of chromium trioxide was added in portions to a mixture of 13 ml of pyridine and 40 ml of chloroform. After 1 h, a solution of 5.5 g of stigmasterol acetate (**1b**) (obtained with a yield of 97% by acetylating stigmasterol (**1a**), mp 142-143°C (hexane); lit. [13]: mp 141°C) in 25 ml of chloroform was added. The mixure was stirred at 15°C for 44 h, and then 50 ml of water was added, the precipitate was triturated, filtered off, and washed on the filter with chloroform, after which the organic layer of the filtrate was separated off and the aqueous layer was extracted with chloroform (3 × 50 ml). The combined organic phases were washed successively with 40-ml portions of water, 5% sulfuric acid, and water, and were dried with magnesium sulfate. The organic solvent was evaporated in vacuum, and the residue was chromatographed on a column of alumina with elution by petroleum ether. This gave 2.7 g (49%) of the initial compound (**1b**).

On further elution with petroleum ether–diethyl ether (4:1), 2.2 g of the acetoxyenone (**2b**) was obtained. Yield 39%, mp 191-193°C (hexane). UV spectrum (EtOH, λ_{max}, nm): 236 (ε 13,800). IR spectrum (KBr, ν, cm^{-1}): 1730(AcO), 1680(C=O), 1630(C=C), 1260(AcO). PMR spectrum ($CDCl_3$, ppm, J, Hz): 0.70(3H, s, Me-18), 0.80(Me-26, d, J = 6.0), 0.80(Me-29, t, J = 7.0), 0.85(Me-27, d, J = 6.0), 1.03(Me-21, d, J = 7.0), 1.22(Me-19, s), 2.05(AcO, s), 4.72(H-3α, m, $W_{1/2}$ = 24.0), 5.01(H-22, dd, J_1 = 15.0, J_2 = 8.0), 5.18(H-23, dd, J_1 = 15.0, J_2 = 8.0), 5.70(H-6, d, J = 1.0).

3β-Hydroxy-(24S)-stigmasta-5,22E-dien-7-one (2a). A suspension of 0.40 g of the acetoxyenone (**2b**) in 50 ml of methanol was treated with 0.10 g of potassium carbonate, and the mixture was stirred at room temperature for 21 h. Then 0.1 ml of glacial acetic acid was added to the mixture, the solvent was evaporated in vacuum, and the residue was chromatographed on a column of silica gel with elution by petroleum ether–ethyl acetate (1:1). This gave 0.28 g of the hydroxyenone (**2a**). Yield 78%, mp 162-164°C (hexane). UV spectrum (EtOH, λ_{max}, nm): 239 (ε 14,600). IR spectrum (KBr, ν, cm^{-1}): 3440(OH), 1670(C=O), 1630(C=C). PMR spectrum ($CDCl_3$, ppm, Hz): 0.70 (Me-18, s), 0.80 (Me-26, d, J = 6.0), 0.80(Me-29, t, J = 7.0), 0.85(Me-27, d, J = 6.0), 1.03(Me-21, d, J = 7.0), 1.20(Me-19, s), 3.68(H-3α, m, $W_{1/2}$ = 19), 5.01(H-22, dd, J_1 = 15.0, J_2 = 8.0), 5.18(H-23, dd, J_1 = 15.0, J_2 = 8.0), 5.69(H-6, d, J = 1.0).

Reduction of the Acetoxyenone (2b). Over 30 h, 1.8 g of sodium tetrahydroborate was added in portions to a stirred suspension of 5.0 g of the acetoxyenone (**2b**) in a mixture of 100 ml of methanol and 50 ml of dioxane. Another 18 h after the final addition of the reagent, 3 ml of glacial acetic acid was added to the mixture, which was then evaporated to a volume of about 20 ml and was diluted with 100 ml of water. The reaction proucts were extracted with ethyl acetate (4 × 50 ml), the combined organic extracts were dried with magnesium sulfate and evaporated in vacuum, and the residue was chromatographed on a column of silica gel. Elution with petroleum ether–ethyl acetate (9:1) led to the isolation of 4.37 g of a mixture of the hydroxyacetates (**3b**) and (**4b**) epimeric at C-7. The total yield was 87%. Further elution, with petroleum ether–ethyl acetate (1:1), gave 0.32 g of (24S)-stigmasta-5,22E-diene-3β,7β-diol (**3a**). Yield 6.4%. IR spectrum (KBr, ν, cm^{-1}): 3430 (OH). PMR

spectrum ($CDCl_3$, ppm, J, Hz): 0.71(Me-18, s), 0.80(Me-26, d, J = 6.0), 0.80(Me-29, t, J = 7.0), 0.85(Me-27, d, J = 6.0), 1.03(Me-21, d, J = 7.0), 1.05(Me-19, s), 3.55(H-3α, m, $W_{1/2}$ = 24), 3.85(H-7α, br.d, J = 8.0), 5.02(H-22, dd, J_1 = 15.0, J_2 = 8.0), 5.17(H-23, dd, J_1 = 15.0, J_2 = 8.0), 5.29(H-6, br.s). On further elution, 0.19 g (3.8%) of a mixture of diols (**3a**) and (**4a**) was obtained. Yet further elution gave 0.07 g of (24S)-stigmasta-5,22E-diene-3β,7α-diol (**4a**). Yield 1.4%, mp 208-210°C (heptane). IR spectrum (KBr, ν, cm^{-1}): 3600, 3280 (OH). PMR spectrum ($CDCl_3$, ppm, J, Hz): 0.70(Me-18, s), 0.79(Me-26, d, J = 6.0), 0.80(Me-29, t, J = 7.0), 0.85(Me-27, d, J = 6.0), 0.99(Me-19, s), 1.03(Me-21, d, J = 7.0), 3.58(H-3α, m, $W_{1/2}$ = 24), 3.85(H-7β, m, $W_{1/2}$ = 11), 5.01(H-22, dd, J_1 = 15.0, J_2 = 8.0), 5.17(H-23, dd, J_1 = 15.0, J_2 = 8.0), 5.60(H-6, d, J = 5.0).

Hydrolysis of the Acetoxyenones (3b) and (4b). A solution in 300 ml of methanol of 3.87 g of the mixture of acetoxyenones that had been obtained was treated with 1.00 g of potassium carbonate. The reaction mixture was stirred at room temperature for 22 h. Then 1.0 ml of glacial acetic acid was added and the solvent was evaporated in vacuum. The residue was chromatographed on a column of silica gel, with elution by hexane–tetrahydrofuran (2:1). Since the mixture had not been separated, it was subjected to rechromatography under the same conditions. This gave 1.45 g of the 3β,7β-diol (**3a**), 1.95 g of a mixture of diols (**3a**) and (**4a**), and 0.06 g of the 3β,7α-diol (**4a**). The total yield of diols (**3a**) and (**4a**), completely identical with those obtained previously, was 3.46 g (98%).

REFERENCES

1. D. J. Slatkin, J. E. Knapp, P. L. Schiff, Jr., C. E. Turner, and M. L. Moll, Phytochemistry, **14**, 580 (1975).
2. G. Schroeder, M. Rohmer, J. P. Beck, and R. Anton, Phytochemistry, **19**, 2213 (1980).
3. K. P. Cheng, H. Nagano, L. Bang, G. Ourisson, and J. P. Beck, J. Chem. Res. S., No. 9, 217 (1977).
4. H. Nagano, J. P. Poyser, K. P. Cheng, L. Bang, G. Ourissonm and J. P. Beck, J. Chem. Res. S., No. 9, 218 (1977).
5. M. Zander, P. Koch, L. Bang, G. Ourisson, and J. P. Beck, J. Chem. Res. S., No. 9, 219 (1977).
6. K. P. Cheng, L. Bang, G. Ourisson, and J. P. Beck, J. Chem. Res. S., No. 3, 84 (1977).
7. A. Amann, G. Ourisson, and L. Bang, Synthesis, No. 11, 1002 (1987).
8. R. Tan and L. Li, Zhongguo Haigang Yaowu, **12**, No. 1, 1 (1994); Chem. Abstr., **121**, 231147 (1994).
9. C. E. Heltzel, A. A. L. Gunatilaka, D. G. I. Kingston, G. A. Hoffmann, and R. K. Johnson, J. Nat. Prod., **57**, No. 5, 620 (1994).
10. N. V. Kovganko and Zh. N. Kashkan, Khim. Prir. Soedin., 771 (1990).
11. N. V. Kovganko and E. A. Éismont, Izv. Akad. Nauk Belarusi, Ser. Khim. Nauk, No. 4, 57 (1993).
12. W. G. Dauben, M. Lorber, and D. S. Fullerton, J. Org. Chem., **34**, No. 11, 3587 (1969).
13. L. Fieser and M. Fieser, Steroids, Reinhold, New York (1959) [Russian translation Mir, Moscow (1964), p. 369].