TRANSFORMED STEROIDS.

CXXVII. SYNTHESIS OF 16α,17α-ISOPROPYLIDENEDIOXY-PREGN-4-EN-11β, 21-DIOL-3,20-DIONE, AN INTERMEDIATE PRODUCT OF THE PREPARATION OF DECANIDE, TRIAMCINOLONE, AND TRIAMCINOLONE ACETONIDE

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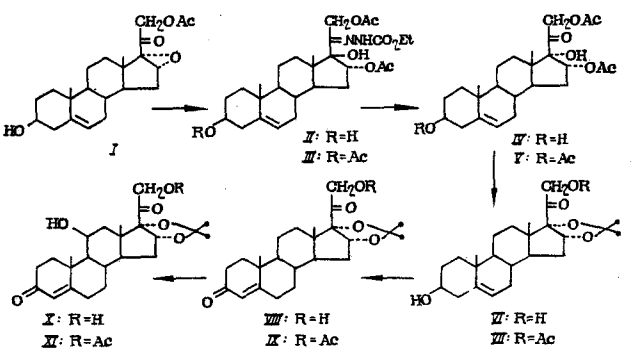
It was shown previously in [1, 2] that opening of the 16α,17α-oxide ring of 20-hydrazones of steroids affords the possibility of synthesizing 16α,17α-dihetero-substituted compounds with atoms of sulfur and nitrogen in position 17.

Continuing our investigations in this area we studied the behavior of the carbethoxyhydrazone grouping on introducing it into 21-acetoxy-20-ketones and removing it, and we determined the possibility of synthesizing decanide, 16α,17α-isopropylidenedioxypregna-1,4-diene-11β,21-diol-3,20-dione, which is a known glucocorticoid preparation with antiinflammatory action [3]. The 21-acetate of 16α,17α-epoxypregn-5-en-3β,21-diol-20-one (I) served as starting material. It was established previously in [4] that opening of the 16α,17α-oxide ring of 20-hydrazones in a series of 21-acetoxy(hydroxy) substituted steroids proceeds significantly more slowly than in the case of the 21-unsubstituted compounds. As a result of finding appropriate conditions, completion of the reaction of oxide (I) with carbethoxyhydrazine and AcOH was successfully achieved after 24 h and the 16,21-diacetate of pregn-5-en-3β,16α,17α,21-tetraol-20-one 20-carbethoxyhydrazone (II) was formed in good yield. The free ketone diacetate (IV) was obtained by removing the hydrazone protection with acetylacetone. Treatment of diacetate (IV) with a mixture of acetone and CH_3OH in the presence of $HClO_4$ led to 16α,17α-isopropylidenedioxypregn-5-en-3β,21-diol-20-one (VI). By selective acetylation at the 21-hydroxyl group [5], Oppenauer oxidation, and saponification of the 21-acetate grouping, the acetonide (VI) was converted in small yield into 16α,17α-isopropylidenedioxypregn-4-en-21-ol-3,20-dione (VIII).



Attempts to oxidize acetonide (VI) directly by Oppenauer without previous protection of the 21-hydroxyl group were unsuccessful. This conversion was effected successfully by microbiological dehydrogenation with cultures of *Corynebacterium mediolanum* [6, 7]. Microbiological hydroxylation of acetonide (VIII) with cultures of *Curvularia lunata* under the usual conditions [8] led to high yields of 16α,17α-isopropylidenedioxypregn-4-en-11β,21-diol-3,20-dione (X) differing from decanide only in the absence of the Δ^1 bond. Since the conversion of (X) into decanide has already been described in the literature [3] as has the

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conversion of the 11β-hydroxy group into a 9α-fluoro-11β-hydroxy group [9, 10] the latter reactions were not carried out.

The studied sequence of reactions therefore may be proposed for the synthesis of the glucocorticoid preparations decanide, triamcinolone, and triamcinolone acetonide and also for the 16α(17α)-thia- and aza analogs of the compounds mentioned and their intermediates.

EXPERIMENTAL

Melting points were determined on a PTP instrument. IR spectra were taken in a Perkin-Elmer 577 IR spectrophotometer in Nujol. To check the course of a reaction and the purity of the obtained substances TLC was applied on microplates with a bound layer of Silufol UV-254 silica gel.

20-Carbethoxyhydrazone of Pregn-5-en-3β,16α,17α,21-tetraol-20-one 16,21-Diacetate (II). Carbethoxyhydrazine (1 g) was added to a suspension of 16α,17α-epoxypregn-5-en-3β,21-diol-20-one 21-acetate (I) (1.3 g) in glacial AcOH (39 ml) and the mixture was stirred at 20-25°C for 24 h. The reaction mixture was poured into water (390 ml), the precipitated solid was filtered off, washed with water to neutral reaction, and dried. Compound (II) (1.5 g: 84%) having mp 199-201°C (from acetone–C_6H_{14}) and $[\alpha]^{20}$ −96° (c 0.5, $CHCl_3$) was obtained. IR spectrum, ν, cm^{-1}: 1230, 1250, 1520, 1625, 1720, 3270, 3490.

Pregn-5-en-3β,16α,17α,21-tetraol-20-one 16,21-Diacetate (IV). A suspension of (II) (1 g) in a mixture of glacial AcOH (50 ml), acetylacetone (4 ml), and water (8.7 ml) was heated at 85-95°C for 1 h, cooled to 20-25°C, and poured into cooled water (590 ml). The precipitated solid was filtered off, washed with water to neutral reaction, and dried. Compound (IV) (0.776 g: 92%) was obtained having mp 204-206°C (from acetone–C_6H_{14}), $[\alpha]_D^{20}$ −60° (c 0.5, $CHCl_3$). IR spectrum, ν, cm^{-1}: 1250, 1710, 1730, 3380, 3500.

16α,17α-Isopropylidenedioxypregn-5-en-3α,21-diol-20-one (VI). A. A suspension of (IV) (0.57 g) in a mixture of acetone (5.7 ml) and CH_3OH (5.7 ml) containing 57% $HClO_4$ (0.35 ml) was heated at 40-42°C for 6 h. After the usual treatment compound (VI) (0.461 g: 90%) was obtained having mp 218-220°C (from CH_3OH), $[\alpha]_D^{20}$ 0° (c 1, $CHCl_3$) [5]. IR spectrum, ν, cm^{-1}: 1710, 3370.

B. A suspension of (V) (0.48 g) in a mixture of acetone (4.8 ml) and CH_3OH (4.8 ml) containing 57% $HClO_4$ (0.6 ml) was stirred at 40-42°C for 6 h. After the usual treatment (VI) (0.362 g: 91%) was obtained having mp 219-221°C (from CH_3OH) and giving no depression of melting point in a mixing test with a known specimen.

20-Carbethoxyhydrazone of Pregn-5-en-3β,16α,17α,21-tetraol-20-one 3,16,21-Triacetate (III). Compound (II) (0.225 g) was acetylated by the usual procedure in a mixture of pyridine (2 ml) and Ac_2O (2 ml) at 20-25°C for 20 h. Compound (III) (0.2 g: 82%) was obtained having mp 185-187°C (from acetone-C_6H_{14}), $[\alpha]_D^{20}$ −98° (c 1, $CHCl_3$). IR spectrum, ν, cm^{-1}: 1225, 1240, 1260, 1630, 1715, 3150, 3250, 3540.

16α,17α-Isopropylidenedioxypregn-5-en-3β,21-diol-20-one 21-Acetate (VII). A solution of (VI) (0.23 g) in pyridine (2.3 ml) and Ac_2O (0.06 ml: 1.1 eq) was maintained at −10°C for 20 h and then poured into cooled water (25 ml). The solid was filtered off, washed with water, and dried. Crude (VII) (0.22 g) was obtained containing 15-20% initial (VI) and traces of the corresponding diacetate in addition to the main product. After recrystallization from CH_3OH (VII) (0.1 g: 39.4%) was obtained having mp 213-215°C [5]. IR spectrum, ν, cm^{-1}: 1235, 1725, 1750, 3450.

16α,17α-Isopropylidenedioxypregn-4-en-21-ol-3,20-dione (VIII). A. A suspension of (IX) (0.2 g) in a mixture of acetone (2 ml) and CH_3OH (2 ml) containing 57% $HClO_4$ (0.3 ml) was heated for 6 h at 40-42°C, then cooled to 20°C and poured into cooled water (40 ml). The precipitated solid was filtered off, washed with water, and dried. Compound (VIII) (0.15 g: 83%) was obtained having mp 237-239°C (from CH_3OH), $[\alpha]_D^{20}$ +134° (c 0.5, $CHCl_3$) [5]. IR spectrum, ν, cm^{-1}: 1610, 1675, 1705, 3500.

B. A culture of *Corynebacterium mediolanum* B-964 [6] was incubated for 30 h at 33°C on solid PS nutrient medium of the following composition: peptone (4.5 g), yeast extract (3 g), casein hydrolyzate (4 g), meat-peptone bouillon (150 ml), glucose (1 g), agar-agar (30 g), water to 1 liter pH 6.9-7.0. Then accumulation of seed material uniform in morphological character was effected in flasks of capacity 500 ml with CM-6 nutrient medium (100 ml) of composition yeast autolyzate (6.0 g), glucose (3.0 g), $K_2HPO_4 \cdot 3H_2O$ (2.5 g), $Na_2HPO_4 \cdot$

$12H_2O$ (4.0 g), NaCl (2.0 g) pH 7.2-7.3 at 33°C for 2 passages of 24 h growth on a shaker (220 rpm). The seeding dose for the first passage was 0.5 vol.% and for the second 0.6 vol.%. The culture from the second passage (7.5 vol.%) was seeded into VK-4K medium (100 ml) of the following composition: corn extract (10 g), $Na_2HPO_4 \cdot 12H_2$ (4.5 g), KH_2PO_4 (1 g) pH 7.0-7.1. After incubation at 33°C for 2 h on a shaker (220 rpm) when the culture was at the beginning of a logarithmic phase of development [7] (VI) (0.2 g) in dimethylformamide (DMF) (2 ml) was introduced. At the end of the transformation (after 16-18 h) the culture fluid was extracted three times with ethyl acetate (100 ml). The extract was washed with water and evaporated to dryness in vacuum. The crystalline residue was dried to constant weight. Compound (VIII) (0.16 g: 80%) was obtained having mp 242-245°C (from MeOH) and gave no depression of melting point in a mixing test with a known specimen.

16α,17α-Isopropylidenedioxypregn-4-en-21-ol-3,20-dione 21-acetate (IX). A. Compound (VIII) (0.15 g) was acetylated by the usual method in a mixture of Ac_2O (1.5 ml) and pyridine (2 ml). Compound (IX) (0.16 g: 96%) was obtained of mp 246-248°C (from CH_3OH) [4]. IR spectrum, ν, cm^{-1}: 1230, 1615, 1670, 1730, 1755.

B. Toluene (about 2 ml) was distilled from a solution of (VII) (0.1 g) in toluene (9 ml) and cyclohexanone (1.2 ml) then aluminum isopropoxide (0.12 g) in toluene (6 ml) was added and the reaction mixture boiled for 18 h with slow distillation of solvent (to 1/3 volume) and adding fresh toluene up to the initial volume. Aluminum isopropoxide (0.12 g) and cyclohexanone (1.2 ml) were added twice to the reaction mixture (after 6 and 12 h). The reaction mixture was cooled, the solid filtered off, and washed with toluene. The combined filtrates were treated with 50% AcOH (pH 3.0) (3 ml), the solid which separated was filtered off, the toluene layer was removed, and the aqueous layer was extracted twice with benzene (about 3 ml). The combined organic extracts were washed twice with $NaHCO_3$ solution, with water, and then evaporated in vacuum. The residue was treated with hexane, the solid product was filtered off, and dried. Technical (IX) (0.09 g: ~90%) was obtained which after recrystallization from CH_3OH had mp 245-248°C and gave no depression of melting point in a mixing test with a sample.

16α,17α-Isopropylidenedioxypregn-4-en-11β,21-diol-3,20-dione (X). A culture of *Curvularia lunata* F-70 [8] was incubated for 7 d at 28°C in solid nutrient medium TO2-D of the following composition: yeast extract (4 g), brewing wort (7%) (200 ml), glucose (4 g), agar-agar (30 g) and water to 1 liter pH 6.9. Accumulation of vegetative mycelium homogeneous in morphological characteristics was then effected in flasks of capacity 500 ml with nutrient medium N (100 ml) of composition sucrose (30 g), yeast autolyzate (2.5 g), $NaNO_3$ (2 g), $(NH_4)_2HPO_4$ (3 g), K_2HPO_4 (1 g), KCl (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g) pH 6.0-6.1 for three successive passages of 30, 24, and 24 h growth respectively at 28°C on a shaker (120 rpm). The seeding dose, comprising 0.5 mycelium from the mass for the first passage, 3 vol. % for the second, and 7 vol. % for the third, was first pulverized mechanically. After the third passage the mycelium was filtered off from nutrient medium and washed with pH 6.0 phosphate buffer. The moist mycelium (60 g) was suspended in the same buffer (300 ml) in a flask of capacity 750 ml and (VIII) (0.15 g) in DMF (3 ml) added. After transformation for 20-24 h at 28°C on a shaker (220 rpm) the culture fluid was filtered and the filtrate was extracted with ethyl acetate (300 ml three times). The extract was washed with water and evaporated to dryness. The residue (0.17 g) was dissolved in acetone (0.5 ml), treated with active carbon, and the solid filtered off. The filtrate was evaporated in vacuum at 20-25°C and the residue dried. Compound (X) (0.14 g: 89.7%) was obtained having mp 203-205°C (from aqueous CH_3OH) [9]. IR spectrum, ν, cm^{-1}: 1620, 1660, 1720, 3430.

16α,17α-Isopropylidenedioxypregn-4-en-11β,21-diol-3,20-dione (XI). Compound (X) (0.16 g) was acetylated by the usual method in a mixture of Ac_2O (1.6 ml) and pyridine (2 ml). Compound (XI) (0.13 g: ~74%) was obtained having mp 241-243°C (from CH_3OH) [9]. IR spectrum, ν, cm^{-1}: 1240, 1620, 1660, 1730, 1750, 3370.

LITERATURE CITED

1. A. A. Akhrem, Z. I. Istomina, A. V. Kamernitskii, et al., Izv. Akad. Nauk SSSR, Ser. Khim., 426 (1974).
2. A. V. Kamernitskii (Kamernitzky), A. J. Terekhina, and I. V. Vesela, Indian J. Chem., 15B, 99 (1977).
3. L. Mantica, R. Ciceri, J. P. Cassagne, et al., Arzneim. Forsch., 20, 109 (1970).
4. A. A. Akhrem, A. V. Kamernitskii, and I. G. Reshetova, Izv. Akad. Nauk SSSR, Ser. Khim., 1364 (1972).

5. P. Crabbe, H. J. Ringold, and J. A. Zderic, Bull. Soc. Chim. Belg., 70, 271 (1961).
6. A. N. Konyakhin, G. A. Trenina, B. I. Demchenko, et al., Khim.-farm. Zh., No. 8, 66 (1977).
7. V. I. Tropina, N. N. Sukhareva-Nemakova, B. I. Demchenko, et al., Mikrobiologiya, 50, 255 (1981).
8. G. A. Trenina, A. N. Konyakhin, L. N. Lavrova, et al., Khim.-farm. Zh., No. 3, 111 (1978).
9. West German Patent No. 1917027 (1969); Chem. Abstr., 72, 44024b (1970).
10. U.S. Patent No. 3338926 (1967); Chem. Abstr., 66, 95308f (1967).

MACROKINETICS OF CHEMICAL REACTIONS IN THE PRESENCE OF IMMOBILIZED ENZYMES

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In recent years we have noticed a substantial interest in the use of immobilized homogeneous catalysts and enzymes by chemists and technologists. Numerous studies [1] have shown that enzymes or homogeneous catalysts (metal complexes in particular) immobilized on a solid carrier surface retain their catalytic properties and possess certain advantages typical for heterogeneous catalytic systems.

The immobilized catalyst activity and selectivity are known to be dependent substantially on the method of its fixation and on the carrier nature [2]. When porous carriers are used, chemical conversion of the substrate on the catalyst includes mass transfer, resulting in the transport of substrate molecules inside the porous carrier and the product molecules out of the pores. Under specific conditions, diffusion seems to affect the rate of the total process and to lower the efficiency of an enzyme or a metal complex immobilized on the inner surface of the porous carrier.

According to the concept of correlation between the outer diffusion rate and the activity of the outer surface of a solid catalyst particle [3], the outer diffusion mode of the process can exist for an immobilized catalyst at low velocities of the medium relative to the particle, thus controlling the rate of substrate diffusion to the outer surface of the catalyst.

When the flow rate is sufficiently high, the mass transfer coefficient of an outer carrier surface unit is substantially greater than the rate constant of the reaction on the outer surface. In other words, the outer kinetic mode of the process is established. In this case, the substrate concentration in the pore openings can be considered as equal to that in the core of the liquid or gaseous phase stream.

After the outer kinetic mode is established further increase in the activity of the solid catalyst is related to the use of the inner surface of the pores with enzyme immobilized thereon, the concentration of the latter being η (g/cm^2). The substrate transport into a pore is described by the diffusion rate r_D, given by the equation

$$r_D = F \cdot D \cdot \frac{dC}{dx}, \tag{1}$$

where F is the pore cross-sectional area (cm^2); D is the apparent diffusion coefficient (sec^{-1}); C is the substrate concentration (mole/liter); and x is the distance from a pore opening (cm).

The substrate diffusing into a pore is converted into the reaction products under the effect of the catalyst in the pore. In the stationary mode, therefore, the mass transfer rate is equal to the rate of the chemical reaction. The Michaelis–Menten equation describes the enzyme reaction kinetics:

$$-\frac{dC}{d\tau} = \frac{k'C}{K_M + C}, \tag{2}$$

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