UDC 547.92, 576.852.1,577.158

STEREODIRECTIVITY OF THE REDUCTION OF 20-OXOSTEROIDS BY ACTINOMYCETES

E. A. Elin, Leonid M. Kogan, N. T. Zelkova, and I. V. Torgov

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 3, pp. 145-149, 1969

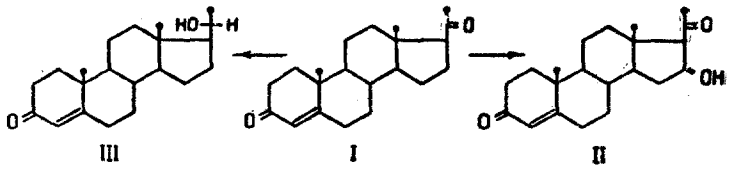
A common reaction in the action of microorganisms on steroids is the reduction of the 20-oxo group of pregnane compounds [1]. In this process, the majority of microorganisms studied previously form 20β-hydroxysteroids, while the reduction of 20-oxosteroids to 20α-alcohols is found only in the case of Saccharomyces cerevisiae (Δ^{16}-dehydro-progesterone yields the product of a retropinacolone rearrangement containing a 20α-hydroxy group [2], Rhodotorula longissima [3,4], Rh. glutinis [5], Penicillium sp. [6], Bacillus megatherium [7], and Cylindrocarpon radicicola [8].

Microbiological reduction of 20-oxosteroids is distinguished by high stereodirectivity (only one epimer of the 20-alcohol is ever formed). In 1966, Kogan et al. [9] observed that Actinomyces roseochromogenus is capable of reducing some 17-oxygen-containing pregnenes to the corresponding 20α-alcohols.

We have attempted to find whether the capacity of A. roseochromogenus for reducing 20-oxosteroids to 20α-alcohols is unique or whether other species of actinomycetes which effect this conversion exist.

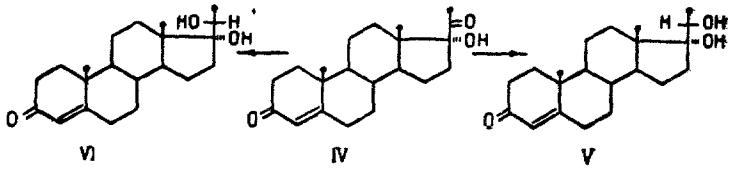
Cultures of actinomycetes were grown on a nutrient medium containing starch and mineral salts. To the growing culture was added an alcoholic solution of the steroid under investigation, and fermentation was carried out for three days. After the usual treatment, samples of the culture mass were chromatographed on micro plates coated with silica gel [10], with authentic samples of the corresponding metabolites as markers. To show up the steroids, the chromatograms were sprayed with sulfuric acid and heated. The 20-epimers with similar or identical chromatographic mobilities were distinguished by heating the chromatograms with Lugol's reagent (see Experimental).

In the first place, we tested actinomycetes capable, like A. roseochromogenus, of hydroxylating progesterone (I) with the formation of 16α-hydroxyprogesterone (II). It was found that all actinomycetes (24 strains of



III I II

20 species) of this group on fermentation with 17α-hydroxyprogesterone (IV) reduced it to 17α,20α-hydroxypregn-4-en-3-one (V) (table).



VI IV V

Eight strains of actinomycetes of this group, on fermentation with 17α-hydroxyprogesterone (IV), formed not only the 20α-alcohol (V) but also its 20β-epimer (VI). Only A. olivaceus and A. viridis were capable of simultaneously hydroxylating progesterone (I) in position 16α and reducing it, with the formation of 20β-hydroxypregn-4-en-3-one (III). These species of actinomycetes are also distinguished by the fact that they are incapable of transforming 17α-hydroxyprogesterone (IV).

Another group of actinomycetes investigated includes those reducing progesterone (I) to 20β-hydroxypregn-4-en-3-one (III). The fermentation of cultures of these microorganisms (23 strains, 19 species) with 17α-hydroxyprogesterone (IV) shows that they all reduce this substrate to 17α,20β-dihydroxypregn-4-en-3-one (VI), and 10 strains form not only the 20β-alcohol (VI) but also its 20α epimer (V).

Thus, a definite relationship is found: actinomycetes that hydroxylate progesterone in position 16α, as a rule, contain an enzyme reducing 17α-hydroxyprogesterone to the corresponding 20α-alcohol. Consequently, it may be predicted with a high degree of probability that species of actinomycetes other than those that we have studied which perform the 16α-hydroxylation of progesterone will, on fermentation with 17α-hydroxyprogesterones, lead to the formation of the 20α-alcohol.

Results obtained show that the capacity of reducing 20-oxopregnenone compounds into their 20α-dihydro derivatives, previously known for only a few microorganisms, is characteristic of many species of actinomycetes. The 20α-alcohol is formed only on fermentation with 17α-hydroxyprogesterone (IV), of the two substrates that we studied.

As previously shown [19], for the reduction of a 20-oxo to a 20α-hydroxy group by a culture of A. roseochromogenus, the molecule of the steroid substrate must contain a 17-oxygen function. This requirement apparently extends also to the transformation of 20-oxopregnenes by other actinomycetes [not one of the actinomycetes used in the experiment reduced progesterone (I) to the 20α-alcohol].

Apart from the metabolites mentioned, many of the cultures give other products of the transformation of progesterone and 17α-hydroxyprogesterone but these are formed in considerably smaller amounts and we have not yet identified them (see table). No transformations of steroids by cultures of A. caprae, A. streptomycini, A. flavus, A. venezuelae, A. gibsonii, A. cinnamonensis, A. flaveolus, A. celluloseae, A. citreus, A. gongerotti, A. intermedius and A. odorifer have previously been described.

The results obtained for some species of actinomycetes differ from those of other investigators in relation to the capacity of these organisms for transforming steroids. Thus, for example, Vondrova and Čapek [11] found no steroid metabolites from the action of cultures of A. celluloseae, A. citreus, A. griseolus, A. intermedius, A. odorifer, A. flavus and A. venezuelae on progesterone.

However, these authors carried out the fermentation of the actinomycetes with progesterone on a nutrient medium with a composition differing substantially from ours, which explains the different nature of the transformation. Moreover, the strains of the microorganisms are not mentioned in the Czech workers' paper, and it is not excluded that the difference in the behavior of organisms of a given species may be due to the fact that we used other strains of the same species.

In favor of the latter assumption are the results of fermentation of cultures of A. annulatus, A. chrysomallus, and A. sulfureus with progesterone on a nutrient medium (analogous in composition to that of Vondrova and Čapek), which do not differ from the results that we obtained in the main series of experiments on a starch medium.

Experimental

To obtain the seed material, 500-ml flasks each containing 100 ml of nutrient medium [2 g of $(NH_4)_2SO_4$, 1 g of $MgSO_4$, 1 g of NaCl, 3 g of $CaCO_3$, 1 g of K_2HPO_4, and 10 g of starch in 1 l of mains water; pH before sterilization 7.0] were inoculated with the air-dry mycelium of the actinomycetes from slope agar and were then cultivated on a shaking machine (200 rpm) at 28° C for 3 days. Flasks each containing 100 ml of the medium described were inoculated with the seed material so obtained (10 ml each). The cultures were grown for 24 hr and then to each flask was added a solution of 10 mg of a steroid in 0.5 ml of ethanol and fermentation was carried out under the conditions described above. Samples (5 ml) were taken 48 and 72 hr after the addition of the steroid. Each sample was extracted with 5 ml of chloroform and the extract was concentrated in vacuum and chromatographed on micro plates with a fixed layer of silica gel [10] in the ether–benzene (1 : 1) system (in the case of fermentation with progesterone) or in ether (in the case of fermentation with 17α-hydroxyprogesterone).

The chromatographic behavior of progesterone, 17α-hydroxyprogesterone, and their 20-dihydro derivatives can be judged from the following information:

Substance	R_f, system	Color on treatment with Lugol's reagent
Progesterone (I)	0.40 ether–benzene (1 : 1)	Yellow
20α-Hydroxypregn-4-en-3-one	0.25 ether–benzene (1 : 1)	Blue
20β-Hydroxypregn-4-en-3-one (III)	0.25 ether–benzene (1 : 1)	Yellow
17α-Hydroxyprogesterone (IV)	0.50 ether	
17α,20α-Dihydroxypregn-4-en-3-one (V)	0.23 ether	Blue
17α,20β-Dihydroxypregn-4-en-3-one (VI)	0.30 ether	Yellow

To detect the steroids, the chromatograms were sprayed with sulfuric acid and heated and were then treated with a 0.3% solution of I_2 in 5% KI solution (Lugol's solution). The results of the fermentation are given in the table.

Cultures of A. annulatus, A. chrysomallus, and A. sulfureus were also fermented with progesterone on a nutrient medium containing 1% of peptone, 1% of glucose, and 0.5% of NaCl in mains water (pH 7.0) under the conditions described above. All three cultures formed 20β-hydroxypregn-4-en-3-one (III) from progesterone (I).

The sample of 20α-hydroxypregn-4-en-3-one was kindly given to us by Dr. M. Garnik (Ikafarm, Israel) and the cultures of actinomycetes were supplied by G. K. Skryabin (Institute of Microbiology, Academy of Sciences of the USSR) and V. D. Kuznetsov (All-Union Scientific Research Institute for Antibiotics, Ministry of Health of the USSR).

Actinomycetes converting progesterone	Reduction of 17α-hydroxyprogesterone to the		Number of other fermentation products in the transformation of	
	20α-alcohol	20β-alcohol	progesterone	17α-hydroxyprogesterone
B Into 16α-hydroxyprogesterone				
A. bikiniensis ATCC 11062	+		6	2
A. californicus WKS	+			
A. californicus ATCC 3312	+		1	1
A. fimicarius IPV-902	+		1	2
A. gelaticus ATCC 3323	+			
A. praecox ATCC 3374	+			
A. vinaceus USSR RIA-591	+			
A. vinaceus NCJB-8852	+			
A. vinaceus NRRL B-2285	+			
A. roseochromogenus ATCC 3347	+			
A. gibsonii ATCC 6852	+			
A. caprae IPV-206	+			
A. streptomycini	+			
A. parvus IFO-3380	+			2
A. lipmanii ATCC 3331	+		4	1
A. aureus ATCC 3309	+	+	1	
A. flavus ATCC 3369	+	+		
A. cinnamonensis IAW	+	+		
A. venezuelae ATCC 3534	+	+		
A. microflavus ATCC 3332	+	+	6	2
A. microflavus IAW	+	+	4	1
A. purpureochromogenus ATCC 3343	+	+	1	
A. olivaceus ATCC 11626*				
A. viridis IPV-894*				
B Into 20β-hydroxypregn-4-en-3-one				
A. bobiliae ATCC 3310	+	+		
A. tendae EIH-14077	+	+		
A. flaveolus IPV-869	+	+		
A. flaveolus ATCC 3319	+	+		
A. flaveolus WKS	+	+		
A. caeruleus Bu-3,7	+	+	1	
A. mediocidicus EIH-24350	+	+	4	2
A. cellulosae ATCC 3313	+	+		
A. citreus WKS	+	+		
A. verne WKS	+	+		
A. verne ATCC 3353		+		
A. coelicolor ATCC 3355		+		
A. chrysomallus ATCC 3657		+		
A. griseolus ATCC 3325		+	1	
A. rutgersensis ATCC 3350		+		
A. annulatus ATCC 3307		+	2	
A. diastaticus ATCC 3315		+	2	3
A. gongerotii ATCC 10975		+		
A. intermedius ATCC 3329		+		
A. odorifer ATCC 6246		+		
A. albus ATCC 3006		+		
A. sulfureus ATCC 3007		+	1	

* 20β-Hydroxypregn-4-en-3-one is formed in addition to 16α-hydroxyprogesterone.

Conclusions

1. It has been established that the reduction of the 20-oxo group of steroids to a 20α-hydroxy group, previously described only for a few microorganisms, is carried out by many actinomycetes.

2. A correlation has been found between the action of the actinomycetes studied on progesterone and the steric directivity of the reduction of 17α-hydroxyprogesterone by these cultures.

REFERENCES

1. A. A. Akhrem and Yu. A. Titov, Microbiological Transformations of Steroids [in Russian], Moscow, 1965.
2. B. Camerino and R. Modelli, Gazz. Chim. Ital., 86, 1219, 1956.
3. F. Carvajal, O. F. Vitale, M. J. Gentles, H. L. Herzog, and E. B. Hershberg, J. Org. Chem., 24, 695, 1959.
4. V. M. Chang and D. R. Idler, Can. J. Biochem. and Physiol., 39, 1277, 1961.
5. T. Takahashi and Y. Uchibori, Agr. Biol., Chem. Tokyo, 26, 89, 1962; C. A., 56, 14739, 1962.
6. D. H. Peterson, S. H. Eppstein, P. D. Meister, H. C. Murray, H. M. Leigh, A. Weintraub, and L. M. Reineke, J. Am. Chem. Soc., 75, 5768, 1953.
7. N. N. Suvorov, L. V. Sokolova, V. M. Ryzhkova, and G. G. Dvoryantseva, DAN SSSR, **152**, 1130, 1963.
8. O. El-Tayeb and S. E. Knight, Ch. J. Sih. Biochim. Biophys. Acta, **33**, 402, 1964.
9. L. M. Kogan and E. A. Yelin, 11 Int. Congr. Horm. Ster., Milano, Abstr., 233, 1966.
10. I. I. Zaretskaya, L. M. Kogan, O. B. Tikhomirova, and I. V. Torgov, KhPS [Chemistry of Natural Compounds], **2**, 321, 1966.
11. O. Vondrova and A. Čapek, Folia Microbiol., **8**, 117, 1963.

27 July 1967

Institute of the Chemistry of Natural Compounds,
AS USSR