OXIDATION OF STEROIDS BY MICROÖRGANISMS. II.[1] HYDROXYLATION IN POSITION 11 AND SYNTHESIS OF CORTISONE FROM REICHSTEIN'S COMPOUND S

Sir:

The finding[1] that the action of the actinomycete culture MD-2428 on progesterone could under proper conditions bring about hydroxylation of the latter at carbon atom 16, a position not readily attacked by chemical oxidants, made it appear hopeful that microörganisms could be found capable of introducing oxygen into the important position 11, and a successful experiment of this sort has recently been reported.[2] As a result of a systematic study of the metabolic action of a variety of microorganisms on selected steroidal substrates we have independently found that certain members of the genus *Aspergillus* are capable of effecting the desired change in practicable yields. In this communication we wish to report our experience with *Aspergillus niger* (Wisc. 72-2) using progesterone, Reichstein's Compound S, desoxycorticosterone and 17α-hydroxyprogesterone as the substrates.

All fermentations were carried out in submerged culture at 25° for periods of 65–70 hours in a medium containing corn steep liquor solids, ammonium phosphate, calcium carbonate and soybean oil. Incubation of *A. niger* with progesterone followed by extraction of the culture filtrate with chloroform yielded a mixture separable by chromatography on alumina into 11α-hydroxyprogesterone[2] (I), 35% yield, m.p. 166–167°; $[\alpha]^{22}D$ +178° (*c*, 1.22 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 241 mμ (ϵ = 17,000); $\lambda_{max.}^{Nujol}$ 2.91μ (OH), 5.85μ (20-keto), 5.97μ (Δ^4-3-keto); (*Anal.* Found: C, 76.12; H, 9.28) and 6ξ,11α-dihydroxyprogesterone (II), 20% yield, m.p. 250–253°; $[\alpha]^{23}D$ +100° (*c*, 0.28 in $CHCl_3$); $\lambda_{max.}^{alc.}$ 236 mμ (ϵ = 15,000); $\lambda_{max.}^{Nujol}$ 2.98μ (OH), 5.89μ (20-keto), 6.04μ (Δ^4-3-keto); (*Anal.* Found: C, 72.66; H, 8.76). In harmony with the data of Peterson and Murray,[2] I formed a monoacetate, m.p. 172–174°; $[\alpha]^{23}D$ +155° (*c*, 0.38 in $CHCl_3$) and on oxidation with chromic acid furnished the known 11-ketoprogesterone,[3] m.p. 170–172°, $[\alpha]^{22}D$ +276° (*c*, 0.29 in $CHCl_3$), +229° (*c*, 0.52 in acetone); identical in all respects with an authentic sample prepared according to Reichstein and Fuchs.[3] II formed a diacetate[2] m.p. 154–155°; $[\alpha]^{23}D$ +81° (*c*, 0.92 in $CHCl_3$) and on oxidation with chromic acid afforded what appears to be 6,11-diketoprogesterone, m.p. 144–146°; $[\alpha]^{23}D$ +143° (*c*, 0.87 in $CHCl_3$); (*Anal.* Found: C, 73.77; H, 7.66) on the basis of its ultraviolet spectra in alcohol ($\lambda_{max.}^{alc.}$ 249 mμ (ϵ = 11,500)) and methanolic KOH ($\lambda_{max.}$ 255 mμ and 370 mμ (ϵ = 9800 and 8900 resp.)), identical with those shown by 6-ketoprogesterone, and of the difference of its molecular rotation and that of 6-ketoprogesterone ($\Delta[M]D$ +407°),[4] indicative of the presence of the 11-keto group in the tetraketone derived from II.

Oxidation of Compound S with *A. niger* afforded two new compounds, Δ^4-pregnene-11α,17α,21-triol-3,20-dione[5] (III), 25% yield, m.p. 217–219°; $[\alpha]D$ +117°, (*c*, 0.46 in alcohol); $\lambda_{max.}^{alc.}$ 242 mμ (ϵ = 15,000); (*Anal.* Found: C, 69.46; H, 8.39); diacetate: m.p. 206–208°; $[\alpha]^{22}D$ +117° (*c*, 0.84 in $CHCl_3$) and an as yet unidentified isomer of III (IV), 15% yield, m.p. 248–250°; $[\alpha]^{23}D$ +97° (*c*, 0.50 in alcohol); $\lambda_{max.}^{alc.}$ 241 mμ (ϵ = 16,000); (*Anal.* Found: C, 69.45; H, 8.53), separable by fractional crystallization from acetone. III was converted by chromic acid to Δ^4-androstene-3,11,17-trione (andrenosterone),[6] m.p. 221–225°; $[\alpha]^{24}D$ +284° (*c*, 0.51 in $CHCl_3$) identical in all respects (including infrared spectra) with an authentic sample of that substance. Acetylation of III with 1.1 moles of acetic anhydride in pyridine followed by chromic acid oxidation furnished in 70% yield cortisone acetate,[7] m.p. 243–244° (opaque at 85–95°); $[\alpha]^{23}D$ +168° (*c*, 0.63 in acetone), +204° (*c*, 0.63 in $CHCl_3$), identical in its physical characteristics with an authentic sample. This sequence of reactions provides unambiguous proof for the structure of III and represents the final steps of a new and simple synthesis of cortisone from readily accessible starting materials.

Incubation of *A. niger* with desoxycorticosterone furnished after chromatography on silica a 67% yield of the hitherto undescribed Δ^4-pregnene-11α,17α-diol-3,20-dione (V), m.p. 153–154°; $[\alpha]^{23}D$ +168° (*c*, 0.7 in $CHCl_3$), (*Anal.* Found: C, 72.90; H, 8.76). The structure of V was established by acetylation with 1 mole of acetic anhydride followed by oxidation with chromic acid to the known 11-dehydrocorticosterone acetate,[8] m.p. 178–179°, $[\alpha]^{23}D$ +239° (*c*, 0.5 in $CHCl_3$), identified by comparison with an authentic sample.

The action of *A. niger* on 17α-hydroxyprogesterone produced two new hydroxylated steroids separable by chromatography on silica. Elution with 5% acetone in chloroform afforded 17a-methyl-D-homo-Δ^4-androstene-11α,17a-diol-3,17-dione[9] (VI), 25%, m.p. 261–262°; $[\alpha]^{23}D$ +46° (*c*, 0.74 in $CHCl_3$); (*Anal.* Found: C, 72.92; H, 8.84), and subsequent elution with 10% acetone in chloroform yielded 11α,17α-dihydroxyprogesterone (VII), 15%, m.p. 219–221°; $[\alpha]^{23}D$ +87° (*c*, 0.57 in $CHCl_3$); (*Anal.* Found: C, 73.11; H, 8.70); monoacetate: m.p. 205–208°; $[\alpha]^{24}D$ +65° (*c*, 0.61 in $CHCl_3$). Oxidation of VI and VII with chromic acid afforded respectively the hitherto undescribed 17a-methyl-D-homo-Δ^4-an-

(1) Preceding communication: D. Perlman, E. Titus and J. Fried, THIS JOURNAL, **74**, 2126 (1952).

(2) D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952). The microbiological conversion of progesterone into 11α-hydroxyprogesterone and an unidentified dihydroxyprogesterone by the fungus *Rhizopus arrhizus* is described. *Cf.* also the more recent publication by D. R. Colingsworth, M. P. Brunner and W. J. Haines (*ibid.*, **74**, 2381 (1952)) reporting the oxidation of Compound S to 17α-hydroxycorticosterone in low yield by *Streptomyces fradiae*.

(3) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).

(4) The contribution for the 11-keto group in 11-ketoprogesterone is +302° ($CHCl_3$).

(5) III was found to be inactive by Dr. R. W. Bates in the rat liver glycogen assay (M. L. Pabst, R. Sheppard and M. H. Kuizenga, *Endocrinology*, **41**, 55 (1947)) in doses of 800 mcg. per animal.

(6) T. Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936).

(7) T. Reichstein, *ibid.*, **20**, 978 (1937); L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

(8) T. Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937).

(9) That the formation of the D-homosteroid VI was effected by the mold and not by one or more of the constituents of the fermentation medium was shown by the fact that incubation of 17α-hydroxyprogesterone with the medium in the absence of the organism caused no expansion of ring D.

drostene-17a-ol-3,11,17-trione, m.p. 238–242°; $[\alpha]^{23}D$ +121° (*c*, 0.48 in $CHCl_3$) and the known Δ^4-pregnene-17α-ol-3,11,20-trione,[10] m.p. 232–235°; $[\alpha]^{24}D$ +186° (*c*, 0.33 in $CHCl_3$). The conversion of VII into VI under conditions reported[11] to effect the expansion of ring D in 17α-hydroxyprogesterone served to establish the structure of VI.

(10) L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948); T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

(11) J. van Euw and T. Reichstein, *Helv. Chim. Acta*, **24**, 879 (1941).

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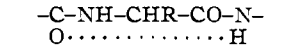
POLYPEPTIDE HELICES IN PROTEINS

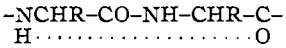
Sir:

About fifteen years ago[1] I discussed the principles underlying protein structure and proposed that the polypeptide chains in proteins, when not nearly fully extended, have folded or helical structures, with adjacent folds or turns of the helix connected by N–H · · · O hydrogen bonds. Considerable evidence has since accumulated in favor of these proposals and they are now generally accepted.

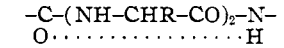
As the simplest examples illustrating these principles, I discussed a folded structure containing 7-atom rings



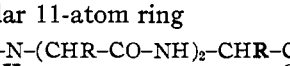
and helices containing 8-atom rings



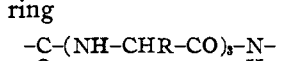
and 10-atom rings



Bragg, Kendrew and Perutz[2] have recently considered similar 11-atom ring



and 13-atom ring



helices, assuming in both exactly four amino-acid residues per turn, and Pauling, Corey and Branson[3] have advocated the 13-atom ring helix with about 3.7 residues per turn. They pointed out, as I had done in the case of the 10-atom ring structure, that it is not necessary that this number be integral. (At the recent Chemical Conclave I mistakenly believed and stated that their model was merely a refinement of my 10-atom ring structure.)

(1) M. L. Huggins, Abstracts, Rochester Meeting, American Chemical Society, B10 (1937); see also Abstracts, Memphis Meeting, A.C.S., P4 (1942); *Annual Review of Biochemistry*, **11**, 27 (1942); *Chem. Revs.*, **32**, 195 (1943).

(2) W. L. Bragg, J. C. Kendrew and M. F. Perutz, *Proc. Roy. Soc. (London)*, **A203**, 321 (1950).

(3) L. Pauling and R. B. Corey, THIS JOURNAL, **72**, 5349 (1950); *Proc. Nat. Acad. Sci.*, **37**, 235, 241, 256, 261, 282 (1951); L. Pauling, R. B. Corey and H. R. Branson, *ibid.*, **37**, 205 (1951).

An 11-atom ring structure is possible,[4] consistent with the published X-ray data and with all of Pauling and Corey's postulates regarding bond angles and distances, except that the N–C* bond is not in the C–C'O–NH plane, but makes an angle of about 30° with it. This is not unreasonable, on the basis of their estimate of about equal contributions of structures containing coplanar nitrogen and tetrahedral nitrogen. On the other hand, approximate coplanarity has been found in glycylglycine[5] and acetylglycine[6] crystals; this would seem to favor the 13-atom ring structure, which permits such coplanarity. However, since the energy difference associated with the difference in bond orientation is probably small and may be counteracted by environmental differences, this evidence is not very strong.

In neither the 11-atom ring structure nor the 13-atom ring structure is the C=O bond tilted with respect to the axis of the helix more than the N–H bond, unless the assumptions made are considerably in error. Hence, the infrared spectrum differences, tentatively and cautiously attributed by Bamford and co-workers[7] to such a difference in angle of tilt, should probably be interpreted in some other way.

In agreement with Bamford and his colleagues, I believe that, pending further experimental data, both of these structures should be considered possible for the alpha synthetic polypeptides, the alpha fibrous proteins and corpuscular proteins. Perhaps both types are sometimes present together, in fibrous natural proteins for example. All other types of structure seem to be definitely eliminated, at least for the alpha synthetic polypeptides, by the X-ray data.[7–9]

(4) M. L. Huggins, THIS JOURNAL, **74**, 3963 (1952).

(5) E. W. Hughes and W. J. Moore, *ibid.*, **71**, 2618 (1949).

(6) G. B. Carpenter and J. Donohue, *ibid.*, **72**, 2315 (1950).

(7) C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Nature*, **169**, 357 (1952).

(8) M. F. Perutz, *ibid.*, **167**, 1053 (1951); **168**, 653 (1951); H. E. Huxley and M. F. Perutz, *ibid.*, **167**, 1054 (1951).

(9) W. Cochran and F. H. C. Crick, *ibid.*, **169**, 234 (1952).

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COÖRDINATES OF THE 11-ATOM RING POLYPEPTIDE HELIX

Sir:

In order to facilitate comparison of the 11-atom ring helical polypeptide structure[1,2] with other structures and with experimental data, I have calculated atomic coördinates, on the following assumptions: (1) the translational and rotational shifts per amino-acid residue are 1.47Å. and 100°, as observed[2–4] in poly-(methyl glutamate); (2) the bond distances and bond angles are those assumed by Pauling and Corey,[5] except that some

(1) M. L. Huggins, THIS JOURNAL, **74**, 3963 (1952).

(2) C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Nature*, **169**, 357 (1952).

(3) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 241 (1951); *Nature*, **169**, 494 (1952).

(4) M. F. Perutz, *ibid.*, **167**, 1053 (1951).

(5) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 235 (1951).