tions in two solvents. The viscosity results confirmed that the intrinsic viscosity of the arachin was increased and the ultracentrifugal examination gave evidence that cross-linking of molecules had occurred as a result of the treatment.

Experimental

The arachin fraction of peanut protein was prepared by extracting defatted peanut meal with 10% (w./v.) sodium chloride and precipitating the arachin at 32% saturation with ammonium sulfate. Purification of the arachin was achieved by a similar reprecipitation. After removing the ammonium sulfate by dialysis, the protein was dried with alcohol and acetone.

The terephthalyl dichloride was supplied by Imperial Chemical Industries, Ltd., Dyestuffs Division. Buffer salts and urea were A.R. or of equivalent purity.

Ultracentrifuge measurements were made in a Spinco model E ultracentrifuge at 50,700 r.p.m. (190,000 g.). Viscosity measurements were made in an Ostwald viscometer at 25°.

For the preparation of the modified arachin, 1.6 g. of terephthalyl dichloride per 20 g. of protein was used, the reaction being carried out at *p*H 9.5 and 0°. These conditions were found by Mann[1] to give the maximum intrinsic viscosity of the modified protein. After the reaction, the protein solution was dialyzed with stirring against phosphate buffer (ionic strength $I = 0.2$, *p*H 7.8) for 48 hours. No attempt was made to estimate the amount of terephthalyl dichloride which had reacted with the protein.

Results and Discussion

In Table I the intrinsic viscosities $[\eta]$ (deciliters per gram) of native and modified arachin in two solvents are shown.

TABLE I

Solvent	η Native arachin	η Modified arachin
Phosphate buffer $I = 0.2$, *p*H 7.8	0.055	0.094
Phosphate buffer $I = 0.2$, *p*H 7.8-7.2 *M* urea	.23	.37

Although Mann's viscosities were measured in 10 *M* urea and the values above obtained in 7.2 *M* urea, there does not appear to be as large an increase attained in this work as was obtained by Mann.

Figure 1 (a) shows the sedimentation diagram of native arachin in phosphate buffer (ionic strength $I = 0.2$, *p*H 7.8) while Fig. 1(b) gives the sedimentation diagram for the modified arachin in the same solvent. From the presence of the faster sedimenting peaks in Fig. 1(b) it is evident that some type of aggregation of the arachin molecules has occurred. (The alternative possibility that the increased sedimentation constant is the result of combination of terephthalyl groups with the protein without cross-linking is unlikely. Mann's figures showed that the maximum terephthalic acid content was 0.052 g. per g. of protein which would be insufficient to increase the sedimentation constant to the values shown.)

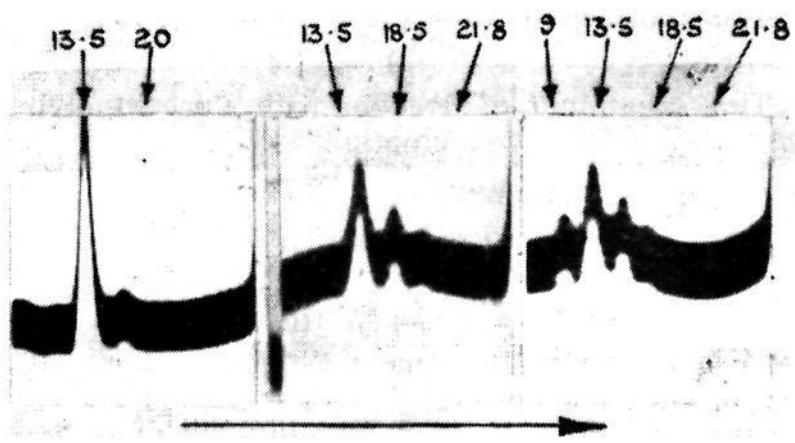

Fig. 1.—Sedimentation diagrams of (a) native arachin, (b) arachin treated with terephthalyl dichloride, (c) arachin treated with terephthalyl dichloride showing dissociated component; in phosphate buffer $I = 0.2$, *p*H 7.8, protein concn. *ca.* 1 g./100 ml. Numbers above peaks refer to rounded sedimentation constants in Svedberg units.

In the absence of diffusion data, accurate determinations of molecular weights are impossible. If, however, a value of 1.3 is assumed for the frictional ratio (f/f_0) then the molecular weights corresponding to the sedimentation constants 13.5, 18.5 and 21.8 of Fig. 1(b) are in the ratio 320,000:500,000:660,000 or very approximately 2:3:4. The value of 500,000 which is *ca.* 1.5 times the molecular weight of arachin could be arrived at if the arachin dissociated into halves at *p*H 9.5 (which it does readily, *cf.* Johnson and Shooter[2]) and three of the dissociated molecules were then linked together with terephthalyl dichloride. In some cases the dissociation product was visible in the ultracentrifuge diagram (Fig. 1(c)).

(2) P. Johnson and E. M. Shooter, *Biochim. Biophys. Acta*, **5**, 361 (1950).

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The C-20 Epimers of 4-Pregnene-11β,17α,20-triol-3-one

BY G. I. POOS

RECEIVED APRIL 21, 1955

Among various metabolites of the adrenal hormones, a substance recently detected on paper chromatograms by DeCourcy, Bush, Gray and Lunnon[1] appears to hold particular biological interest. Excretion of this substance in the urine of non-pregnant females was reported[1] to reach a high level in the week prior to menstruation. The material also could be detected in the urine of pregnant women, although in appreciably lower concentration. On the basis of R_f values and color reactions, the authors advanced the structure 4-pregnene-11β,17α,20-triol-3-one for the new metabolite.

As an aid in the identification of the compound, authentic samples of 4-pregnene-11β,17α,20-triol-3-one, both in the 20β V and 20α VIII stereoisomeric modification, have been synthesized. It is the purpose of the present communication to present a synthetic route to V and VIII, and also to record the physical properties of the pure crystalline materials.

Although a variety of methods are available for the synthesis of the 4-pregnene-11,17,20-triol-3-ones, a route involving lithium aluminum hydride reduction of the 3-dioxolane of 21-desoxycortisone

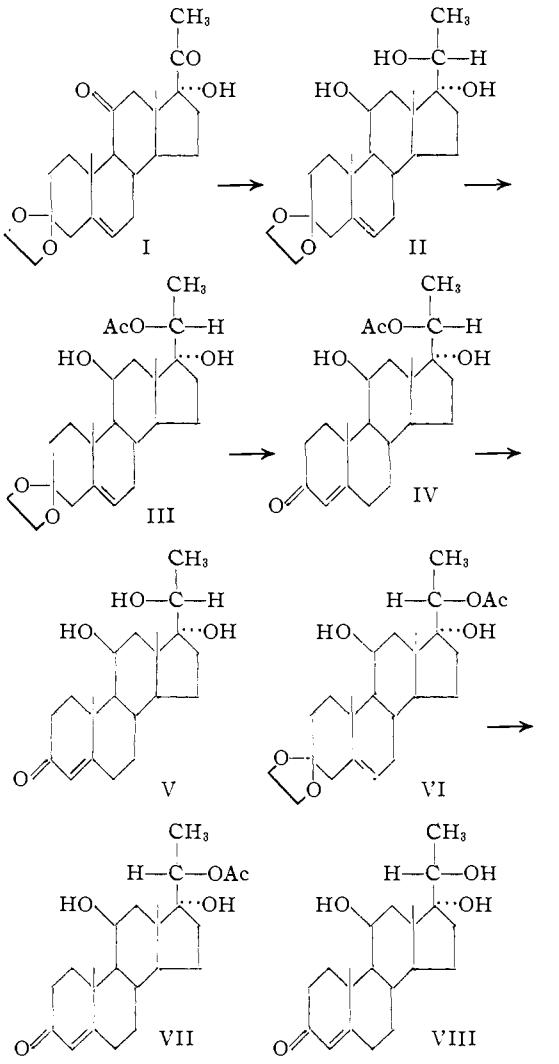
(1) C. DeCourcy, I. E. Bush, C. H. Gray and J. N. Lunnon, *J. Endocrinology*, **9**, 401 (1953).

(I) was chosen because of the ease of preparation of I. Thus the 3-dioxolane of cortisone acetate[2] can be saponified, converted to the 21-mesylate and thence to 3-ethylenedioxy-21-iodo-5-pregnene-17α-ol-11,20-dione in good yield.[3] Hydrogenolysis of the latter over palladium–barium carbonate proceeded quantitatively to the 3-dioxolane of 21-desoxycortisone. The iodo ketone also was reduced by boiling with Raney nickel in ethanol but the yield of I by this method was less satisfactory (50–60%). The 21-desoxypregnene I never was obtained with a sharp melting point, apparently due to thermally induced D-homo rearrangement.[4] Evidence for homogeneity of the material was obtained by acid hydrolysis of a sample of the crude hydrogenolysis product which afforded the known 21-desoxycortisone[5] in good yield.

Lithium aluminum hydride reduction of I proceeded to give non-crystalline material which was subjected to chromatography on Florisil. Two clearly defined fractions were obtained consisting of 75% of a less-polar and 20% of a more-polar material. The more-polar product was acetylated with acetic anhydride in pyridine and yielded a crystalline mixture from which 4% (over-all) of a single compound was separated with the correct analysis for a 3-ethylenedioxy-5-pregnene-11,17,20-triol diacetate. This evidence suggests that the more-polar product consisted largely of a mixture of the 20-isomerides of the 11α-hydroxypregnene and that the proportion of 11α-hydroxy product encountered in this case was somewhat higher than has been observed previously in the lithium aluminum hydride reduction of steroid 11-ketones. This reduction has been discussed recently by Levin, *et al.*[6]

The 75% of less-polar reduction product proved to be a mixture of the desired 11β-hydroxy-20-isomerides. Isolation of the pure epimers proved to be tedious and at no point was it possible to effect a clean-cut separation. Early fractions of the Florisil chromatogram crystallized and by repeated recrystallization, 12% (over-all) of a single product was isolated. This material proved to be the 11β,17α,20β-triol II. An attempt to hydrolyze the 3-dioxolane of II with *p*-toluenesulfonic acid in acetone proceeded anomalously.[7] Acetylation of II with acetic anhydride in pyridine proceeded as expected and gave the 20-monoacetate III. With *p*-toluenesulfonic acid in acetone, III was hydrolyzed in the normal manner to 3-keto-Δ^4-monoacetate IV. Compound IV was saponified with aqueous methanolic potassium carbonate to triol V. Pure, sharp-melting IV could be prepared only by the re-acetylation of V; a small amount of impurity had a marked effect upon the melting point of IV. The structure of triol monoacetate IV was proved by oxidation to the known 4-pregnene-17α,20β-diol-3,11-dione 20-monoacetate.[8] A difference in molecular rotation of +243° was observed between 20β-hydroxy compound V and 20-acetate IV. Both the direction and magnitude of this change are consistent with changes observed in acetylation of an 11-keto-17α,20β-dihydroxysteroid.[9,10]

The mixture of 11β,17α,20-triols which remained after separation of a portion of the 20β-isomer II was acetylated. Repeated crystallization of this mixture of products afforded 13% (from I) of a material that was substantially pure 3-ethylenedioxy-5-pregnene-11β,17α,20α-triol 20-acetate (VI). Acid hydrolysis of VI gave the 20-monoacetate of 4-pregnene-11β,17α,20α-triol-3-one (VII) which upon saponification with carbonate was converted



(2) J. M. Constantin, A. C. Haven and L. H. Sarett, THIS JOURNAL, **75**, 1716 (1953).

(3) F. A. Cutler, F. J. Fisher, H. E. Mertel, R. M. Lukes, J. P. Conbere and L. H. Sarett, *ibid.*, in press.

(4) See for example T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 186 (1951).

(5) L. H. Sarett, *ibid.*, **70**, 1454 (1948).

(6) R. H. Levin, B. J. Magerlein, A. V. McIntosh, A. R. Hanze, G. S. Fonken, J. L. Thompson, A. M. Searcy, M. A. Scheri and E. S. Gutsell, *ibid.*, **76**, 546 (1954).

(7) None of the expected triol V was obtained but rather a compound that appeared by analysis to be an acetonide of V. Several attempts to prove an acetonide structure for this product were unrewarding. A similar situation appears to have been encountered by Adams, *et al.*, *J. Chem. Soc.*, 2299 (1954), who have written a 17,20-acetonide structure for a product obtained from 12-ethylenedioxy-pregnane-3β,17α,20ξ-triol with acetone and *p*-toluenesulfonic acid.

(8) L. H. Sarett, THIS JOURNAL, **68**, 2478 (1946).

(9) L. H. Sarett, *ibid.*, **71**, 1175 (1949).

(10) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, New York, N. Y., 1949, p. 434.

to 4-pregnene-11β,17α,20α-triol-3-one (VIII). The observed ΔM_D of $-65°$ in the conversion VIII → VII is that expected for transformation of a 17α,20α-dihydroxy compound to the corresponding 20-monoacetate[9,10] and provides a reasonable basis for assignment of structure to the isomeric triol VIII.

By acid hydrolysis of the mother liquors from the separation of VI and chromatography of the product, it was possible to isolate an additional 25% (from I) of 20β-isomer IV and 10% of 20α-isomer VII. The combined yield of 11β,17α,20β-triol was 37% while that of the 11β,20α-triol was 23%. It would appear that in this case the proportion of 20α-hydroxy product is somewhat greater than in the lithium aluminum hydride reduction of derivatives of progesterone and 17α-hydroxyprogesterone.[11]

Experimental[12]

3-Ethylenedioxy-5-pregnene-17α-ol-11,20-dione (I).—The hydrogenation of 2.078 g. of 3-ethylenedioxy-21-iodo-5-pregnene-17α-ol-11,20-dione[3] in 300 ml. of ethanol was carried out in the presence of 4.5 g. of 5% palladium-on-barium carbonate under 40 lb. of hydrogen pressure at room temperature overnight. The catalyst was separated by filtration and the filtrate was concentrated to a small volume and diluted with water whereupon the crystalline product precipitated; 1.571 g. (100%), m.p. 183–194°. Recrystallization of this material from various solvents did not give a sharp-melting sample. Thermal D-homo rearrangement seemed to account for the melting behavior. Platelets melting at 190–195° with partial resolidification and melting to 215° were obtained from methanol while recrystallization from ethyl acetate gave rods which slowly changed above 195° to prisms melting at 210–215°. The analytical sample was prepared by successive recrystallizations from methanol and ethyl acetate and melted at 190–195° and 210–215°; λ_{max} 295 mμ, ϵ_{mol} 86; λ_{max} 2.8–2.9, 5.85, 5.92 μ.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.14; H, 8.18.

A 40-mg. sample of the crude product above, m.p. 183–194°, was hydrolyzed by boiling briefly with 10 mg. of *p*-toluenesulfonic acid in 2 ml. of acetone. Precipitation of the product with water gave 32 mg. of 21-desoxycortisone which after recrystallization from ethyl acetate amounted to 22 mg. (62%) and melted at 228–231° alone or when mixed with an authentic sample.[5]

3-Ethylenedioxy-5-pregnene-11β,17α,20β-triol (II).—A solution of 1.32 g. of the ol-dione I and 800 mg. of lithium aluminum hydride in 30 ml. of tetrahydrofuran was heated under reflux for 2.5 hours. The cooled solution was treated dropwise with water and the precipitated inorganic material separated by filtration. Concentration of the filtrate to dryness afforded an amorphous residue which was dissolved in ether–petroleum ether and adsorbed on 60 g. of Florisil. From the early 7:3 ether–petroleum ether eluates there was obtained 600 mg. of crystalline II. Recrystallization from ether and then acetone gave 160 mg. of II which melted at 159–161°. A sample was recrystallized twice from acetone for analysis; m.p. 161–162°, λ_{max} 2.9 μ, $[\alpha]^{24}D$ $-52 \pm 2°$ (*c* 1.00, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.32; H, 9.19.

Further elution with ether and ether–chloroform yielded non-crystalline material which was combined with mother liquors from the purification of crystalline II for acetylation and separation of the 20α-isomer (see below).

Finally, elution with 1:1 chloroform–acetone provided 270 mg. (20%) of a more polar product which could not be induced to crystallize. Acetylation with acetic anhydride–pyridine (100°, 12 minutes) gave a crystalline mixture, m.p. 190–220°. Recrystallization from ethyl acetate and acetone afforded 65 mg. of a diacetate, m.p. 253–254°; λ_{max} 2.80, 5.80, 5.86 μ; $[\alpha]^{23}D$ $-74 \pm 2°$ (*c* 1.13, $CHCl_3$).

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 67.77; H, 8.44.

Acid Hydrolysis of II.—A solution of 135 mg. of II, m.p. 159–161°, in 5 ml. of acetone was treated with 20 mg. of *p*-toluenesulfonic acid and left at room temperature overnight. The reaction mixture was diluted with water and extracted with ether and the washed and dried ether solution was passed over 5 g. of acid-washed alumina. Ether eluates provided 90 mg. of crystals, m.p. 154–159°. Recrystallization from ether–petroleum ether gave a sample with m.p. 158–160°; λ_{max} 242 mμ, ϵ mol 15,900; λ_{max} 2.92, 6.01, 6.13, 9.1 μ; $[\alpha]^{23.5}D$ $+86 \pm 4°$ (*c* 0.45, $CHCl_3$).

Anal. Calcd. for $C_{24}H_{36}O_4$ (acetonide): C, 74.19; H, 9.34. Found: C, 74.28, 74.15; H, 9.35, 9.46.

Acetylation of 3-Ethylenedioxy-5-pregnene-11β,17α,20β-triol.—One hundred sixty milligrams of triol II, m.p. 154–156°, was allowed to stand at room temperature overnight in a mixture of 1.5 ml. of pyridine and 0.8 ml. of acetic anhydride. Addition of ice and water caused separation of 154 mg. of 3-ethylenedioxy-5-pregnene-11β,17α,20β-triol 20-acetate (III), m.p. 202–208°. Recrystallization from ether and acetone–petroleum ether raised the melting point to 208–211°; $\lambda_{max}^{CHCl_3}$ 2.78, 5.78 μ; $[\alpha]^{23}D$ $-6 \pm 2°$ (*c* 0.975, $CHCl_3$).

Anal. Calcd. for $C_{25}H_{40}O_6$: C, 68.77; H, 9.24. Found: C, 68.75; H, 8.74.

Acid Hydrolysis of 3-Ethylenedioxy-5-pregnene-11β,17α,-20β-triol 20-Acetate.—A solution of 87 mg. of III and 20 mg. of *p*-toluenesulfonic acid in 5 ml. of acetone was boiled for 20 minutes. The reaction mixture was diluted with water and the crystalline precipitate was isolated, 72 mg., m.p. 208–225°. Repeated recrystallization of this material from ether, methanol, ethyl acetate and acetone afforded 56 mg. with melting point 230–240° and $[\alpha]^{22}D$ $+175 \pm 2°$ (*c* 1.00, $CHCl_3$). Pure 4-pregnene-11β,17α,20β-triol-3-one 20-acetate (IV) was obtained only by saponification and isolation of the triol V followed by acetylation; it had m.p. 246–249°; λ_{max} 2.87, 5.78, 6.09, 6.19 μ; $[\alpha]^{23}D$ $+171 \pm 2°$ (*c* 1.08, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.98. Found: C, 70.44; H, 8.87.

4-Pregnene-11β,17α,20β-triol-3-one (V).—The triol monoacetate IV (170 mg., m.p. 235–248°) was heated under reflux for 80 minutes in a solution of 10 ml. of methanol and 10 ml. of 1 *N* potassium carbonate. Water was added to the reaction mixture and the methanol was removed under vacuum. After extraction with chloroform and washing, drying and concentration of the chloroform extract there was obtained 150 mg. of crude crystalline product which afforded 125 mg. of 4-pregnene-11β,17α,20β-triol-3-one (V), m.p. 146–149°, by recrystallization from ethyl acetate–petroleum ether. Crystallization from aqueous methanol gave a solvated form melting at 99–101° dec. The analytical sample was recrystallized from ether and melted at 149–151°; λ_{max} 242 mμ, ϵ_{mol} 15,650; λ_{max} 2.85, 2.95, 6.0, 6.1 μ; $[\alpha]^{23}D$ $+122 \pm 2°$ (*c* 1.06, $CHCl_3$).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.49; H, 9.19.

Acetylation of 60 mg. of V (m.p. 148–151°) in acetic anhydride–pyridine at room temperature overnight afforded 44 mg. of pure IV, melting at 246–249° after two recrystallizations from ethyl acetate.

Oxidation of 4-Pregnene-11β,17α,20β-triol-3-one 20-Acetate.—A mixture of 24 mg. of IV, m.p. 246–249°, 25 mg. of chromic anhydride and 0.5 ml. of pyridine[13] was left at room temperature overnight. One milliliter of water was added and the mixture was extracted with five small portions of ether. The ether solution was washed with dilute hydrochloric acid and water and was dried and evaporated to dryness leaving 22 mg. of crystalline product. Recrystallization from ether afforded 16 mg. of 4-pregnene-17α,20β-diol-3,11-dione 20-acetate,[7] m.p. and mixed m.p. 218–220°.

(11) J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, THIS JOURNAL, **73**, 1528 (1951).

(12) Melting points were determined on a Kofler micro hotstage. Ultraviolet spectra were determined in methanol solution; infrared spectra are of the crystalline solids in Nujol unless otherwise noted. The author is indebted to Mr. R. N. Boos and his associates for the microanalyses, to Mr. F. A. Bacher and staff for the ultraviolet spectra and to Mr. R. W. Walker for the infrared spectra.

(13) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

3-Ethylenedioxy-5-pregnene-11β,20α-triol 20-Acetate (VI).—The mixture of C_{20}-epimers remaining from the isolation of II was acetylated in acetic anhydride–pyridine (100°, 12 minutes) and gave 900 mg. of a crystalline mixture. Recrystallization from acetone–ether, acetone and ether provided 198 mg. of VI melting at 177–180° with partial re-solidification and melting at 200°; $\lambda_{max}^{CHCl_3}$ 2.75, 2.85, 2.92, 5.78 μ; $[\alpha]^{22}D$ −56 ± 2° (*c* 1.07, $CHCl_3$).

Anal. Calcd. for $C_{25}H_{40}O_6$: C, 68.77; H, 9.24. Found: C, 68.72; H, 9.15.

Acid hydrolysis (*p*-toluenesulfonic acid in acetone) of the mother liquors from which VI was separated, gave, after chromatography on acid-washed alumina, 350 mg. of the 20β-acetate IV and 140 mg. of the 20α-acetate VII.

4-Pregnene-11β,17α,20α-triol-3-one 20-Acetate (VII).—A solution of VI (97 mg.) in 2.5 ml. of acetone containing 25 mg. of *p*-toluenesulfonic acid was boiled for 15 minutes, diluted with water and the crystalline product was collected; 83 mg., m.p. 208–220°. Recrystallization from methanol, acetone and ethyl acetate provided pure VII melting at 224–226°; λ_{max} 2.9, 5.77, 6.05, 6.19 μ; $[\alpha]^{22}D$ +79 ± 2° (*c* 1.01, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.98. Found: C, 70.71; H, 8.20.

4-Pregnene-11β,17α,20α-triol-3-one (VIII).—To a solution of 148 mg. of VII, m.p. 215–223°, in 8 ml. of methanol was added 4 ml. of 1 *N* potassium carbonate solution. After 16 hours at room temperature the reaction mixture was treated with water and the product was extracted with chloroform. The dried extract was evaporated to dryness and the crystalline residue was recrystallized from ether; yield 112 mg., m.p. 190–193°. Pure VIII was obtained by recrystallization from acetone–petroleum ether; m.p. 193–194°; λ_{max} 242 mμ, ϵ_{mol} 15,900; λ_{max} 2.9–2.95, 6.08 μ; $[\alpha]^{23}D$ +107 ± 2° (*c* 0.550, $CHCl_3$).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.11; H, 9.22.

A subsequent saponification of VII yielded VIII with a melting point of 210–214°. A sample which melted at 193–194° was converted to the higher-melting form by seeding a super-saturated solution.

Acknowledgment.—The author is indebted to Dr. L. H. Sarett for his encouragement and suggestions in connection with this work.

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Occurrence of Choline Sulfate in *Penicillium Chrysogenum*[1]

BY CARL M. STEVENS AND PRAN VOHRA

RECEIVED MAY 9, 1955

The appearance of a note by de Flines[2] prompts us to report more quantitative data obtained earlier on the occurrence of "cyclic" choline sulfate in the mycelium of *Penicillium chrysogenum* (Wis 48-701), and on the utilization of this compound as a source of sulfur for the biosynthesis of penicillin.[3]

Using a mutant strain of *Neurospora crassa*[4] which shows a growth response to choline sulfate as well as to free choline, it was demonstrated that culture filtrates of *P. chrysogenum* grown on a purified medium with S^{35}-labeled sodium sulfate as the sole source of sulfur contained negligible amounts of choline or its derivative, while autolysates of the washed mycelium showed significant choline activity. Choline sulfate was determined quantitatively by addition of synthetic choline sulfate to the autolysates, reisolation and determination of the specific activity of the isolated material. The results indicate approximately 0.2 g. of choline sulfate per 100 g. of dry mycelium.

Hockenhull[5] reported a high content of ethereal sulfate in the mycelium of *P. chrysogenum* (Q-176) and suggested that this material may "act as a reservoir of sulfur for the organism." To test specifically whether added choline sulfate is utilized by the mold, the compound was synthesized from S^{35}-labeled sulfuric acid by the method of Schmidt and Wagner.[6] When added to the culture medium as the sole source of sulfur, the compound supported good growth of the mold. When, however, it was tested against inorganic sulfate in competitive utilization experiments,[7] it was found that the latter was utilized almost exclusively as the source of sulfur for the biosynthesis of penicillin.

Experimental

Procedures for culture of the mold, assay for penicillin, determination of radioactivity, etc., have been previously described.[7] After 72 hours fermentation, the mycelium was collected, washed, a portion retained for moisture determination, and the remainder allowed to autolyse.[8] To the autolysate was added a known amount of choline sulfate. After treatment of the solution with Neuberg's reagent,[8] choline sulfate was reisolated by precipitation with phosphotungstic acid and decomposition of the phosphotungstate,[8] or by direct crystallization from the autolysate after passage through cation-exchange (Amberlite IR-112) and anion-exchange (Amberlite XE-75) columns. In either procedure, the recovered choline sulfate was recrystallized from 95% ethanol to constant radioactivity. Specific activities of the samples indicated a content of 0.2 g. of choline sulfate per 100 g. of dry mycelium. This must be considered a minimum value for the intact mycelium, since it was not determined whether any destruction of choline sulfate occurred during autolysis.

S^{35}-Labeled choline sulfate was prepared from choline chloride and sulfuric acid by the general procedure of Schmidt and Wagner.[6] A solution of 2 g. of dried choline chloride in 5 ml. of concentrated sulfuric acid was heated at

TABLE I

COMPARISON OF UTILIZATION OF SULFATE WITH CHOLINE SULFATE FOR PENICILLIN BIOSYNTHESIS

Compd. added[a]	Yield of penicillin, units/ml. 70 hr.	120 hr.	Radioactivity in extracted penicillin, c.p.s./100 units 70 hr.	120 hr.
S^{35}-Labeled Na_2SO_4	150	275	5.6	5.8
S^{35}-Labeled Na_2SO_4	140	275	5.2	5.1
S^{35}-Labeled Na_2SO_4 + choline sulfate	110	400	4.9	3.6
S^{35}-Labeled Na_2SO_4 + choline sulfate	130	300	5.8	4.7
Na_2SO_4 + S^{35}-labeled choline sulfate	110	325	0.3	0.03
Na_2SO_4 + S^{35}-labeled choline sulfate	140	350	0.3	0.1

[a] Each compound was added in an amount equivalent to 10.9 mg. S. The total radioactivity of added sodium sulfate was 11,200 c.p.s. and of added choline sulfate 1,900 c.p.s. by the counting procedure employed.

(1) This work was supported in part by a grant from Eli Lilly and Company, Indianapolis.

(2) J. de Flines, THIS JOURNAL, **77**, 1676 (1955).

(3) C. M. Stevens, P. Vohra, E. Inamine and O. A. Roholt, Jr., *Federation Proc.*, **12**, 275 (1953).

(4) C. M. Stevens and A. Mylroie, *Am. J. Botany*, **40**, 424 (1953).

(5) D. J. D. Hockenhull, *Biochem. J.*, **43**, 498 (1948).

(6) E. Schmidt and W. Wagner, *Ann.*, **337**, 54 (1904).

(7) C. M. Stevens, P. Vohra, E. Inamine and O. A. Roholt, Jr., *J. Biol. Chem.*, **205**, 1001 (1953).

(8) D. W. Woolley and W. H. Peterson, *ibid.*, **122**, 213 (1937).