[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX S. A.]

Steroids. LXXIV.[1] Synthesis of Δ^4-Pregnene-17α,21-diol-3,20-dione (Reichstein's Substance S) and of $\Delta^{1,4}$-Pregnadiene-17α,21-diol-3,20-dione[2]

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A new and efficient partial synthesis of Δ^4-pregnene-17α,21-diol-3,20-dione (Reichstein's substance S) (VIII) from Δ^5-pregnene-3β,17α-diol-20-one 3-formate (I) is described, which proceeds by successive bromination, treatment with sodium iodide, potassium acetate displacement, C-17 acetylation, Oppenauer oxidation and saponification. Moreover a practical six-step conversion of Δ^5-pregnene-3β,17α,21-triol-20-one 3-formate 17,21-diacetate (V) (an intermediate in the Substance S synthesis) to $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (XIIb) (Δ^1-dehydro substance S) has been accomplished. Several unsuccessful attempts to prepare substance S from derivatives of Δ^5-pregnene-3β,17α-diol-20-one (XIII) *via* novel derivatives of Δ^5-pregnene-3β,17α,21-triol-20-one (XV) are adumbrated.

Δ^4-Pregnene-17α,21-diol-3,20-dione (Reichstein's substance S) (VIII) has recently achieved a position of considerable importance as a substrate for microbiological oxidation at C-11 and consequent conversion to cortisone,[3a] hydrocortisone[3b] and 9-fluorohydrocortisone.[3c] In the previous paper of this series[1] we reported the transformation of 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one (a compound readily obtained from diosgenin in four steps) to Δ^5-pregnene-3β,17α-diol-20-one 3-formate (I) in 76% yield. Utilizing our recently discovered Oppenauer oxidation of Δ^5-3β-ol formates to Δ^4-3-ketones,[1] we have now converted I to substance S (VIII) in nearly 50% yield by a simple six-step sequence which compares favorably as regards yield, availability of starting materials and adaptability to large scale operation to the other known syntheses of VIII.[4] Further, we have devised a synthesis of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (Δ^1-dehydro substance S) (XIIb)[5] from Δ^5-pregnene-3β,17α,-21-triol-20-one 3-formate 17,21-diacetate (V) (an intermediate in our synthesis of substance S), which is superior to the one described earlier from this Laboratory.[5] The Δ^1-dehydro derivatives of the cortical hormones are of special interest at this time in view of the discovery that the introduction of a Δ^1-double bond into cortisone,[6a] hydrocortisone[6a] and 9-fluorohydrocortisone[5,6b] greatly enhances the activity of these hormones.

The first step in the preparation of substance S from I involved the bromination of the latter in anhydrous methylene chloride with 2 molar equivalents of bromine, whereby no attack of the formate grouping occurred and the crystalline 5,6,21-tribromo compound II was obtained. Treatment with sodium iodide in dry acetone produced 21-iodo-Δ^5-pregnene-3β,17α-diol-20-one 3-formate (III) through elimination of bromine at C-5 and C-6 and displacement of bromine by iodine at C-21.[7] Reaction of III with potassium acetate in boiling acetone readily gave Δ^5-pregnene-3β,17α,21-triol-20-one 3-formate 21-acetate (IV), which on treatment with acetic anhydride and *p*-toluenesulfonic acid[8] furnished the corresponding 3-formate 17,21-diacetate V.[9] It was found most convenient in the sequence leading from I to IV not to effect purification of the intermediates, for in this way the triester IV crystallized directly from the reaction mixture in the substantially pure state in nearly 70% over-all yield.

The next step involved direct Oppenauer oxidation of IV by means of aluminum isopropoxide in a mixture of boiling xylene[10] and cyclohexanone, as had the corresponding 21-desoxy derivative,[1] when Δ^4-pregnene-17α,21-diol-3,20-dione diacetate (substance S diacetate) (VII)[8b] was produced in 75% yield. Alternatively the triester IV may first be hydrolyzed selectively at C-3 by means of dilute hydrochloric acid in dioxane at room temperature to Δ^5-pregnene-3β,17α,21-triol-20-one 17,21-diacetate (VI) and the latter substance then subjected to Oppenauer oxidation. This oxidation, however, gave no better yield than did the corresponding oxidation of the triester V and the more direct route to VII is therefore preferred.

Saponification[8b] of substance S diacetate (VII)

(1) Paper LXXIII, H. J. Ringold, B. Löken, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **78**, 816 (1956).

(2) Part of the work described in this paper forms the basis of Mexican Patent Application No. 34216 (Aug. 2, 1952) and No. 35342 (Jan. 13, 1953).

(3) (a) H. C. Murray, D. H. Peterson, *et al.*, U. S. Patent 2,602,769; THIS JOURNAL, **75**, 412 (1953); J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952); (b) G. M. Shull and D. A. Kita, *ibid.*, **77**, 763 (1955); (c) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953); **76**, 1455 (1954).

(4) T. Reichstein and J. v. Euw, *Helv. Chim. Acta*, **23**, 1258 (1940); **24**, 1140 (1941); L. H. Sarett, *J. Biol. Chem.*, **162**, 627 (1946); K. Miescher and J. Schmidlin, *Helv. Chim. Acta*, **33**, 1840 (1950); P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, THIS JOURNAL, **72**, 5145 (1950); B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, *ibid.*, **73**, 189 (1951); J. Heer and K. Miescher, *Helv. Chim. Acta*, **34**, 359 (1951); G. Rosenkranz, C. Djerassi, R. Yashin and J. Pataki, *Nature*, **168**, 28 (1951); O. Mancera, H. J. Ringold, C. Djerassi, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **75**, 1286 (1953); C. Meystre, E. Vischer and A. Wettstein, *Helv. Chim. Acta*, **37**, 1548 (1954).

(5) G. Rosenkranz, J. Pataki, S. Kaufmann, J. Berlin and C. Djerassi, THIS JOURNAL, **72**, 4081 (1950). The same substance has been prepared subsequently by microbiological methods by E. Vischer, C. Meystre and A. Wettstein (*Helv. Chim. Acta*, **38**, 835 (1955)) and by A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg (THIS JOURNAL, **77**, 4184 (1955)).

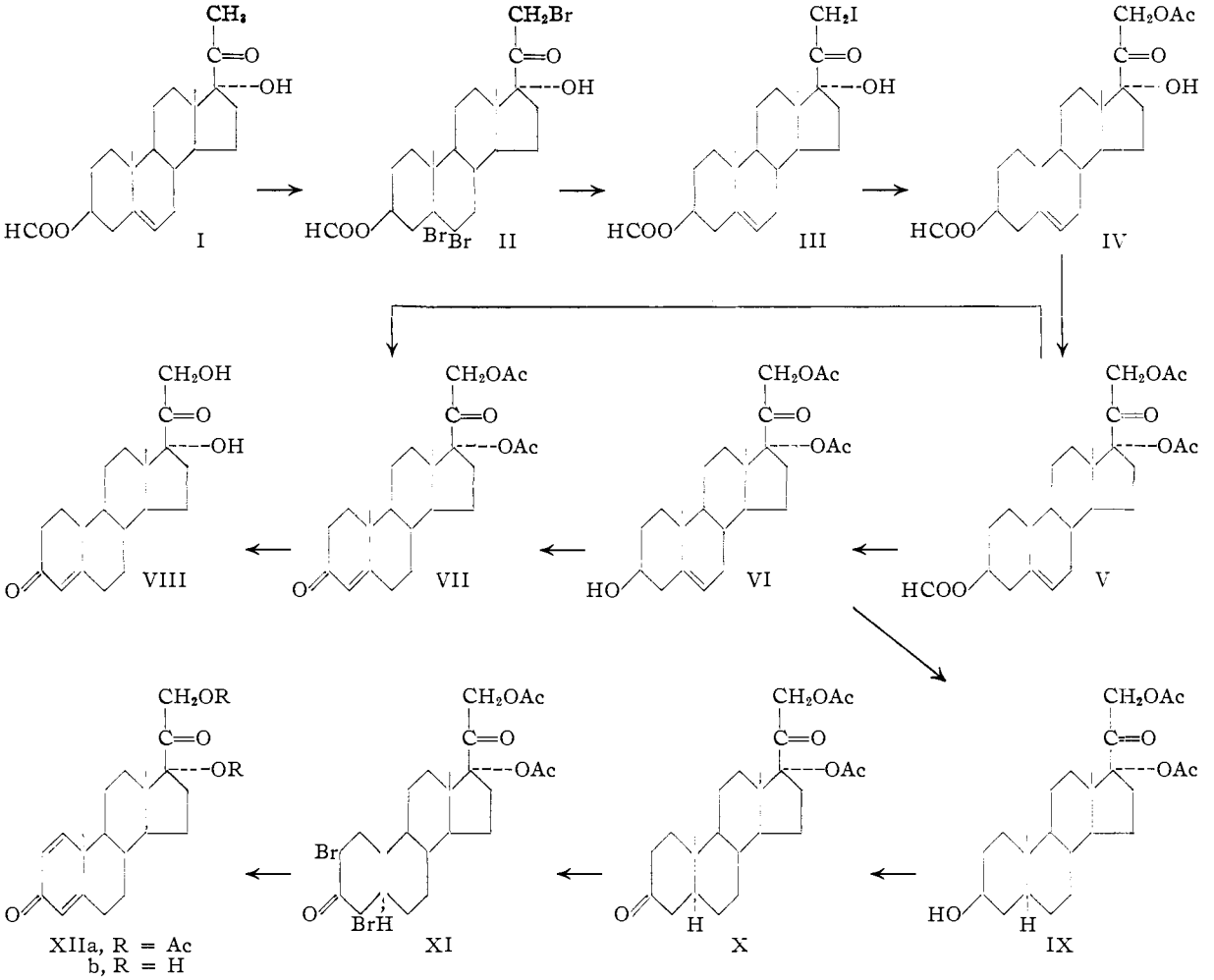
(6) (a) J. J. Bunim, M. M. Pechet and A. J. Bollet, *J. Am. Med. Assoc.*, **157**, 311 (1955); H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955); (b) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler, THIS JOURNAL, **77**, 3166 (1955); J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *ibid.*, **77**, 4181 (1955).

(7) *Cf.* P. L. Julian, *et al.*, reference 4.

(8) *Cf.* (a) Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952); (b) R. B. Turner, *ibid.*, **75**, 3489 (1953).

(9) The acetylation at C-17 was necessary in order that the subsequent Oppenauer oxidation could be carried out without rearrangement in ring D (reference 8b).

(10) See footnote 12 in reference 1.



was best effected through treatment with methanol containing 1.15 molar equivalents of potassium hydroxide at 0–5° for 80 minutes, whereby substance S (VIII) was obtained in 92% yield.[11] The over-all yield from I was 48% and the synthesis has been carried out successfully with identical results on the kilogram scale.

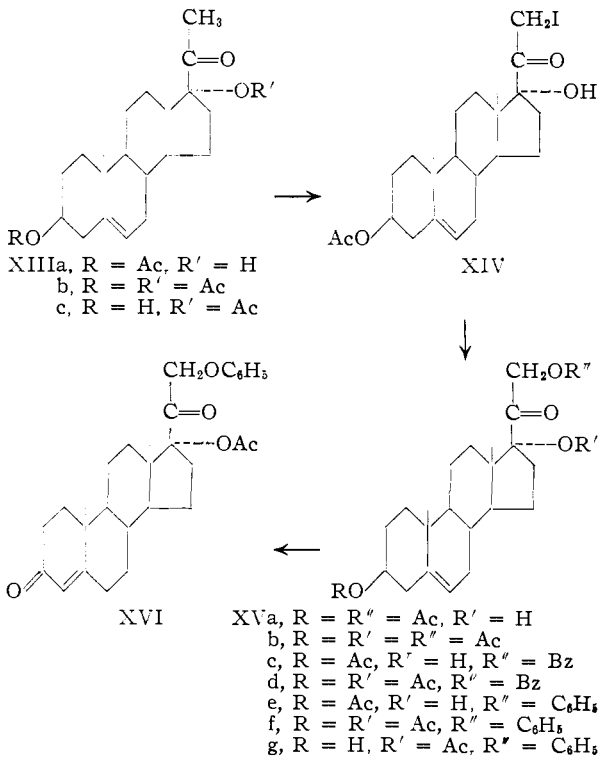
The synthesis of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (XIIb) proceeds from Δ^5-pregnene-3β,-17α,21-triol-20-one 17,21-diacetate (VI), which as mentioned previously is obtainable (in nearly quantitative yield) by acid hydrolysis of the corresponding 3-formate V. Hydrogenation of VI over a palladium–charcoal catalyst led to allopregnane-3β,17α,21-triol-20-one 17,21-diacetate (IX), which on direct oxidation with chromium trioxide in acetic acid produced 90% of allopregnane-17α,21-diol-3,20-dione diacetate (X). Dibromination of X in acetic acid gave 57% of the 2,4-dibromo compound XI, which with a mixture of boiling collidine and lutidine furnished $\Delta^{1,4}$-pregnadiene-17α,-21-diol-3,20-dione diacetate (XIIa) with the expected ultraviolet maximum at 244 mμ (log ϵ 4.16)[12] in 62% yield. It is to be noted that this bromination–dehydrobromination sequence proceeded considerably more smoothly when carried out with the diacetate X than it had with the corresponding 21-monoacetate.[5,13] Finally, saponification of XIIa, as indicated previously for substance S diacetate (VII), yielded $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (Δ^1-dehydro substance S) (XIIb), identified by acetylation to the 21-monoacetate.[5]

Prior to the above-described successful synthesis of substance S by the formate route, a number of unsuccessful routes were investigated which had as their aim the obtention of a derivative of Δ^5-pregnene-3β,17α,21-triol-20-one unsubstituted at C-3 but protected at C-17 and C-21. Oppenauer oxidation and removal of the protecting groups would

(11) This saponification proceeds, at least in part, by ester interchange since less than the theoretical amount (2 molar equivalents) of base were necessary. The ease of hydrolysis of this type of 17,21-diacetate deserves comment in view of the much greater resistance to hydrolysis of C-21 unsubstituted 17α-acetoxy-20-ketopregnane derivatives. Thus we have found that Δ^5-pregnene-3β,17α-diol-20-one diacetate (XIIIb)[5b] is unaffected at C-17 on treatment with methanolic potassium hydroxide (to yield the 17-monoacetate XIIIc almost quantitatively) under conditions which cause complete saponification of the corresponding 21-acetoxy derivative XVb (see Experimental Part). The 21-phenoxy grouping appears to exert a similar influence on the 17α-acetate for saponification of Δ^5-pregnene-3β,17α,21-triol-20-one 3,17-diacetate 21-phenyl ether (XVe) proceeds more readily than of the C-21 unsubstituted compound (despite the fact that the phenyl ether is unaffected) and some preferential saponification of C-17 over C-3 is even observed (*vide infra*).

(12) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953), Table 9.

(13) An alternate synthesis of the diacetate X, involving the direct C-17 acetylation of the 21-monoacetate with acetic anhydride and *p*-toluenesulfonic acid proceeded in less satisfactory yield.



then yield substance S. The most obvious approach to such a derivative appeared to be one involving bromination of Δ^5-pregnene-3β,17α-diol-20-one 17-monoacetate (XIIIc)[8b] to the corresponding 5,6,21-tribromo compound, which on successive treatment with sodium iodide and potassium acetate would be expected to produce Δ^5-pregnene-3β,17α,21-triol-3,20-dione 17,21-diacetate (VI). In fact it was found that the initial bromination did not proceed smoothly since bromination at the C-21 position was very slow and the final potassium acetate displacement could not be brought about at all, even at 100° in a sealed tube.[14]

Another path investigated proceeded from Δ^5-pregnene-3β,17α,21-triol-20-one triacetate (XVb), which was readily prepared from Δ^5-pregnene-3β-17α-diol-20-one 3-acetate (XIIIa)[1] by successive bromination, sodium iodide treatment to XIV, potassium acetate displacement to XVa and C-17 acetylation in the exact manner described above in the 3-formate series. We hoped that a partial hydrolysis of XVb to the corresponding 17-monoacetate could be effected, when preferential C-21 acetylation would yield the 17,21-diacetate VI. However, it was not found possible under a variety of conditions to saponify the C-3 and C-21 acetate groupings without concomitant saponification of the 17-acetate.[11] Similarly Δ^5-pregnene-3β,17α-21-triol 3,17-diacetate 21-benzoate (XVd) (prepared by displacement of 21-iodo-Δ^5-pregnene-3β,-17α-diol-20-one 3-acetate (XIV) with potassium benzoate in ethanol solution to give XVc, followed by C-17 acetylation) could not be saponified at C-3 without saponification at C-17 and C-21.

(14) Similarly I. Salamon and T. Reichstein (*Helv. Chim. Acta*, **30**, 1616 (1947)) were unable to displace the bromo group of a 21-bromo-17β-acetoxyisopregnan-20-one derivative (21-bromo-17-isoallopregnane-3β-diol-20-one diacetate) by the acetoxy function.

Finally we chose the phenyl ether as a means of protecting the 21-hydroxy group. Reaction of 21-iodo-Δ^5-pregnene-3β,17α-diol-20-one 3-acetate (XIV) with potassium phenoxide produced Δ^5-pregnene-3β,17α,21-triol-20-one 3-acetate 21-phenyl ether (XVe), which on C-17 acetylation yielded the corresponding diacetate XVf. Saponification of this substance at 10° with methanolic potassium hydroxide furnished *ca.* 50% of the desired Δ^5-pregnene-3β,17α,21-triol-20-one 17-acetate 21-phenyl ether (XVg) (the C-3 saponification product), together with the corresponding 3-acetate 21-phenyl ether (XVe) (the C-17 saponification product) and starting material. Oppenauer oxidation of XVg led to substance S 17-acetate 21-phenyl ether (XVI), but all attempts to remove the protecting groups from this compound without destruction of the side-chain were unsuccessful.

Experimental[15]

5,6,21-Tribromopregnane-3β,17α-diol-20-one 3-Formate (II).—Δ^5-Pregnene-3β,17α-diol-20-one 3-formate (I) (200 g., m.p. 203–206°)[1] was dissolved in 4 l. of methylene chloride[16] and 200 cc. of solvent was distilled to remove moisture. The solution was cooled to 20° and a solution of 182 g. (2.05 molar equivalents) of bromine in 1.4 l. of methylene chloride was added with stirring under anhydrous conditions, at room temperature, without further cooling (half of the bromine solution was added in three equal portions during 15 minutes and the second half was then added all at once). Bromine uptake was usually complete within 15 to 30 minutes after the end of the addition and a slight vacuum was then applied to remove hydrogen bromide. The solution was washed with sodium bicarbonate solution and water and concentrated to dryness under reduced pressure, the bath temperature not being allowed to exceed 35°. The crude tribromo-compound showed m.p. 143–145° dec. and was used for the following step without delay since it underwent partial decomposition on being allowed to stand. Crystallization from ether–pentane furnished the analytical sample with m.p. 154–156° dec., $[\alpha]_D$ −37°.

Anal. Calcd. for $C_{22}H_{31}O_4Br_3$: C, 44.10; H, 5.22; Br, 40.02. Found: C, 44.54; H, 5.37; Br, 39.51.

21-Iodo-Δ^5-pregnene-3β,17α-diol-20-one 3-Formate (III).—Sodium iodide (360 g.) was added in 3 equal portions at 10-minute intervals to a stirred solution of the crude tribromo-compound II (derived from 200 g. of I) in 2 l. of dry acetone, the temperature not being allowed to exceed 25° by occasional cooling. After being allowed to stand at room temperature for 17 hours, the mixture was diluted with 200 cc. of water and then poured into a solution of 400 g. of sodium thiosulfate in 14 l. of ice-water, with stirring. The precipitate was collected, washed well with water and dried *in vacuo* at 30°. The crude iodo-compound III (271 g.) thus obtained exhibited m.p. 125–135° and was employed for the subsequent step. A small sample was crystallized from methanol with as little heating as possible and then showed m.p. 145–147°.

Anal. Calcd. for $C_{22}H_{31}O_4I$: I, 26.10. Found: I, 26.50.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3-Formate 21-Acetate (IV).—A solution of 271 g. of the crude iodo-compound III in 3 l. of dry acetone was boiled under reflux with 550 g. of anhydrous potassium acetate for 20 hours. The solvent was removed by distillation and the residue was diluted with ice and water. The precipitate was collected, washed well with water and dried at 90°. This procedure furnished 209 g. of the crude diester IV with m.p. 180–185°, which was

(15) Melting points are uncorrected. Rotations were determined (at 20°) in chloroform, and ultraviolet absorption spectra in 95% ethanol solution unless noted otherwise. We should like to thank Mrs. P. Lopez and Miss M. T. Cardenas for these determinations and Mrs. A. Gonzalez for the microanalyses.

(16) The methylene chloride was purified by being washed successively with concentrated sulfuric acid, water and dilute sodium bicarbonate solution. It was then dried over calcium chloride and distilled.

used for the next step. Crystallization of a sample from acetone produced a pure specimen with m.p. 207–209°, $[\alpha]_D$ −16°.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.56; H, 8.26.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3-Formate 17,21-Diacetate (V).—*p*-Toluenesulfonic acid hydrate (66 g.) was added to a suspension of 209 g. of the crude diester IV in 1.5 l. of acetic anhydride and the mixture was stirred for 18 hours at room temperature. A homogeneous solution resulted after a few hours and the product then began to separate. The resulting suspension was cooled in an ice–salt mixture and the precipitate was collected, washed with a small volume of ice-cold acetic anhydride and then with warm water. On being dried, the triester V weighed 168 g. (66% yield from I) and showed m.p. 216–219°. The acetic anhydride mother liquors were diluted with water, stirred to hydrolyze the excess anhydride, the precipitate was collected, washed well with water, dried and crystallized from 200 cc. of acetic anhydride. Another 9.8 g. of V with m.p. 215–218° was thus obtained, the over-all yield from I being thereby increased to 70%. Crystallization of a sample from acetone produced a pure specimen with m.p. 220–222°, $[\alpha]_D$ −65°.

Anal. Calcd. for $C_{26}H_{36}O_7$: C, 67.80; H, 7.88. Found: C, 67.81; H, 8.00.

The same yield of triester V was obtained by heating 100 g. of the crude diester IV with 450 cc. of acetic anhydride containing 3 g. of *p*-toluenesulfonic acid hydrate for 45 minutes at 80–85°.

Δ^5-Pregnene-3β,17α,21-triol-20-one 17,21-Diacetate (VI).—A suspension of 50 g. of the triester V in 1,500 cc. of dioxane, 300 cc. of water and 50 cc. of concentrated hydrochloric acid was stirred at room temperature (25°) for 7 hours. The resulting homogeneous solution was poured into a salt solution containing ice and the precipitate was collected, washed with water and dried. The resulting diacetate VI weighed 45.9 g. (98%) and showed m.p. 196–200°. Crystallization from acetone–hexane led to the analytical sample with m.p. 199–201°, $[\alpha]_D$ −59°. Inferior yields were obtained when the hydrolysis time was either increased or decreased.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.76; H, 8.51.

Δ^4-Pregnene-17α,21-diol-3,20-dione Diacetate (Substance S Diacetate) (VII) (a) By Direct Oppenauer Oxidation of V.—A solution of 200 g. of the triester V in 6.1 l. of commercial xylene[10] and 2.0 l. of cyclohexanone was distilled until 500 cc. of distillate had been collected, in order to remove moisture. Aluminum isopropoxide (210 g.) was added in several portions during 5 minutes to the solution while the latter was being heated to boiling and the mixture was then boiled under reflux for 45 minutes. It was cooled rapidly, ice and water were added and the solvents were removed by steam distillation. The resulting solid was collected by filtration on Celite and dried. Extraction with hot chloroform followed by crystallization from chloroform–methanol produced 141 g. (75%) of substance S diacetate with m.p. 215–219°. The analytical sample was obtained by crystallization from methanol and showed m.p. 220–222°, $[\alpha]_D$ +66°, +52° (dioxane), λ_{max} 240 mμ, log ϵ 4.23; reported[8b] m.p. 220–221°, $[\alpha]_D$ +49.5° (dioxane).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 70.00; H, 7.63.

(b) By Oppenauer Oxidation of VI.—The oxidation of VI was carried out exactly as above, except that an equivalent volume of toluene was substituted for xylene. In this way a 74% yield of substance S diacetate with m.p. 216–219° was obtained, which did not depress the m.p. of the product obtained above.

Δ^4-Pregnene-17α,21-diol-3,20-dione (Substance S) (VIII).—A solution of 15 g. of potassium hydroxide in 100 cc. of methanol, previously cooled to 0°, was added to a suspension of 100 g. of substance S diacetate in 1 l. of methanol at 0° with stirring in a nitrogen atmosphere. The mixture was then stirred at 0–5° for 80 minutes in nitrogen. After 30 minutes a homogeneous solution had resulted, but the product began to crystallize shortly thereafter. Glacial acetic acid (20 cc.) was added, the suspension was concentrated under reduced pressure to *ca.* 250 cc. and was then diluted with an ice-cold aqueous salt solution. The solid was collected and after being washed well with water, dried at 90° and crystallized from ethyl acetate, afforded 74.1 g. (92%) of substance S with m.p. 204–208°. A further purified sample showed m.p. 210–212°, $[\alpha]_D$ +126°, +122° (dioxane), λ_{max} 240 mμ, log ϵ 4.24; reported m.p. 207–209°,[17a] 202–213°,[17b] $[\alpha]_D$ +132°[17b]. Identity with a sample prepared by an independent route was demonstrated by non-depression in m.p. on admixture.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.62; H, 8.70.

Allopregnane-17α,21-diol-3,20-dione Diacetate (X).—A solution of 32 g. of Δ^5-pregnene-3β,17α,21-triol-20-one 17,21-diacetate (VI) in 500 cc. of methanol was shaken in hydrogen with 10 g. of a 5% palladium–charcoal catalyst (American Platinum Works) at 45° and 25 lb. of pressure until uptake of gas ceased (*ca.* 2 hr.). The catalyst was then removed, washed well with hot methanol and the filtrate was evaporated to dryness. The residue (31.6 g.) showed m.p. 184–187° and consisted of crude allopregnane-3β,17α,21-triol-20-one 17,21-diacetate (IX). It was found most efficient to employ this material directly for the oxidation step, without further purification.

The saturated diacetate IX (31.6 g.) was dissolved in 400 cc. of glacial acetic acid and a solution of 9.5 g. of chromium trioxide in 40 cc. of water and 250 cc. of acetic acid was added gradually during 30 minutes, the temperature being kept below 25° by occasional ice cooling. After being allowed to stand at room temperature for 1 hour, the solution was poured into water and the precipitate was collected, washed well with water and dried. Crystallization from acetone–hexane furnished 28.8 g. (90% from VI) of the diketone X with m.p. 234–236°. A further purified sample showed m.p. 238–240°, $[\alpha]_D$ +12°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.42; H, 8.53.

2,4-Dibromoallopregnane-17α,21-diol-3,20-dione Diacetate (XI).—Bromine (16 g.) dissolved in 150 cc. of glacial acetic acid was added during 20 minutes to a stirred solution of 20 g. of the diketone X in 1.5 l. of C.P. acetic acid (containing a few drops of a saturated solution of hydrogen bromide in acetic acid) at room temperature. A precipitate separated during the addition and stirring was continued until a homogeneous solution was again obtained. The reaction mixture was set aside overnight at room temperature under anhydrous conditions and was then poured into ice and water. The product was extracted with methylene chloride and the organic layer was washed with sodium carbonate solution and water. Drying and concentration to a volume of *ca.* 50 cc., followed by dilution with 200 cc. of ether and ice-cooling, produced 15.5 g. (57%) of the dibromo compound XI with m.p. 195–196° (decomp., introduced at 180°). The m.p. was not raised by further crystallization from methanol–chloroform.

Anal. Calcd. for $C_{25}H_{34}O_6Br_2$: C, 50.85; H, 5.80; Br, 27.08. Found: C, 51.26; H, 6.05; Br, 26.81.

Treatment of the mother liquors with zinc in acetic acid at room temperature for 4 hours, followed by chromatographic purification of the product on alumina, resulted in the recovery of 10–20% of the starting material X.

$\Delta^{1,4}$-Pregnadiene-17α,21-diol-3,20-dione Diacetate (XIIa).—A solution of 15.5 g. of the dibromo compound XI in 50 cc. of γ-collidine and 50 cc. of 2,4-lutidine was boiled under reflux for 1 hour with exclusion of moisture. It was then cooled, diluted with ether and the precipitated amine hydrobromide(s) (9.4 g.) were removed and washed with ether. The filtrate was washed with excess 5% hydrochloric acid, water and sodium bicarbonate solution and was then dried and evaporated. Crystallization of the residue from acetone–hexane led to 7.0 g. (62%) of the diene diacetate XIIa with m.p. 187–190°. The analytical specimen showed m.p. 193–194°, $[\alpha]_D$ +29°, λ_{max} 244 mμ, log ϵ 4.16.

Anal. Calcd. for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 70.37; H, 7.30.

$\Delta^{1,4}$-Pregnadiene-17α,21-diol-3,20-dione (Δ^1-Dehydro substance S) (XIIb).—Saponification of 12 g. of the diene diacetate XIIa with methanolic potassium hydroxide was carried out exactly as described above for substance S diacetate VII and yielded, after crystallization from acetone,

(17) (a) T. Reichstein, *Helv. Chim. Acta*, **21**, 1490 (1938); (b) C. Meystre, E. Vischer and A. Wettstein, *ibid.*, **37**, 1548 (1954).

6.2 g. (64%) of the diol XIIb with m.p. 240–242° (introd. at 220°). A further purified sample exhibited m.p. 245–246° (introd. at 220°), $[\alpha]_D$ +74°, λ_{max} 244 mμ, log ε 4.16.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.43; H, 8.26.

Acetylation with acetic anhydride and pyridine (room temperature, overnight) produced the 21-monoacetate with m.p. 224–226°, $[\alpha]_D$ +91°. The m.p. was undepressed on admixture with a sample of the previously described compound[5]; reported[5] m.p. 216–218°, $[\alpha]_D$ +88°.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3,21-Diacetate (XVa).—A solution of 47.0 g. of bromine in 200 cc. of chloroform (J. T. Baker C.P.) was added dropwise during 1 hour to 50 g. of Δ^5-pregnene-3β,17α-diol-20-one 3-acetate (XIIIa)[1] in 400 cc. of chloroform with stirring under anhydrous conditions at room temperature. Stirring was continued for another 10 minutes when decolorization was complete. The solution was washed with water and sodium bicarbonate solution and was then evaporated to dryness under reduced pressure at 30°. The resulting crude 5,6,21-tribromo compound with m.p. 162–167° (decomp.) was dissolved in 1.5 l. of dry acetone, 150 g. of sodium iodide were added in three portions with stirring during 15 minutes and the solution was allowed to stand at room temperature for 18 hours. It was then poured into 4 l. of ice-cold water containing 300 g. of sodium thiosulfate and the resulting crude iodo compound XIV was collected, washed with water and dried at 30° under reduced pressure.

The above iodo compound XIV derived from 50 g. of XIIIa was dissolved in 500 cc. of dry acetone, 200 g. of anhydrous potassium acetate was added and the mixture was boiled under reflux for 17 hours. Removal of most of the acetone followed by water precipitation and crystallization from methanol produced 43.5 g. (75% from XIIIa) of Δ^5-pregnene-3β,17α,21-triol-20-one 3,21-diacetate (XVa) with m.p. 195–197°, $[\alpha]_D$ −9°; reported (J. Heer and K. Miescher[4]) m.p. 195°, $[\alpha]_D$ −13°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.08; H, 8.70.

Δ^5-Pregnene-3β,17α,21-triol-20-one Triacetate (XVb).—The 3,21-diacetate XVa (4.9 g.) was stirred with 2.8 g. of *p*-toluenesulfonic acid hydrate in 250 cc. of acetic anhydride for 17 hours at room temperature. The mixture was then poured into ice and water and after the hydrolysis of the excess anhydride was complete, the precipitate was collected, washed with water and dried. Crystallization from chloroform–methanol furnished 4.85 g. (90%) of the triacetate XVb with m.p. 209–211°, $[\alpha]_D$ −51°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07. Found: C, 68.61; H, 7.77.

Saponification of Δ^5-Pregnene-3β,17α,21-triol-20-one Triacetate (XVb).—The partial hydrolysis at C-3 and C-21 of the 3,17,21-triacetate XVb could not be effected under a variety of conditions (potassium bicarbonate, potassium carbonate and potassium hydroxide in methanol, aqueous tetrahydrofuran and aqueous dioxane). Only the complete saponification will be described.

A suspension of 25 g. of the triacetate XVb in 2 l. of methanol was cooled to 10° and a solution of 25 g. of potassium hydroxide in 500 cc. of methanol, previously cooled to 10°, was added in an atmosphere of nitrogen. The mixture was stirred and allowed to attain room temperature. The resulting homogeneous solution was neutralized with glacial acetic acid 90 minutes after addition of the base, most of the solvent was removed under reduced pressure and water was added to the residue. Crystallization of the resulting precipitate from acetone yielded 14.3 g. (78%) of Δ^5-pregnene-3β,17α,21-triol-20-one with m.p. 230–232°, $[\alpha]_D$ −16°, no acetate band in the infrared; reported (J. Heer and K. Miescher[4]): m.p. 224–226°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.04; H, 9.06.

Partial Saponification of Δ^5-Pregnene-3β,17α-diol-20-one Diacetate (XIIIb) to the 17-Monoacetate XIIIc.—The saponification was carried out with 18.7 g. of the diacetate XIIIb[8b] in 800 cc. of methanol with 18.7 g. of potassium hydroxide in 150 cc. of methanol, as described directly above for the corresponding 21-acetoxy derivative XVb. Crystallization of the crude precipitated product from acetone–hexane furnished 15.1 g. (90%) of the 17-monoacetate XIIIc with m.p. 227–229°, $[\alpha]_D$ −59°, −66° (dioxane); reported[8b] m.p. 230–231.5°, $[\alpha]_D$ −68° (dioxane).

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.79; H, 9.07.

On acetylation of XIIIc with acetic anhydride and pyridine (room temperature, overnight) the original 3,17-diacetate XIIIb was reformed. The 17-acetoxy grouping had therefore not been affected in the saponification step.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3-Acetate 21-Benzoate (XVc).—Δ^5-Pregnene-3β,17α-diol-20-one 3-acetate (XIIIa) (15 g.) was converted to the iodo compound XIV by successive bromination and sodium iodide displacement, as described above for the preparation of the 3,21-diacetate XVa. The total crude iodo compound was dissolved in 200 cc. of absolute ethanol and then boiled under reflux with 50 g. of potassium benzoate and 15 g. of benzoic acid for 20 hours. Most of the solvent was removed under reduced pressure, the residue was poured into sodium bicarbonate solution and the resulting solid was collected, washed with water and dried. Crystallization from chloroform–methanol yielded 6.9 g. (35% from XIIIa) of the 3-acetate 21-benzoate XVc with m.p. 194–197°. The analytical sample showed m.p. 198–200°, $[\alpha]_D$ +30°.

Anal. Calcd. for $C_{30}H_{38}O_6$: C, 72.85; H, 7.74. Found: C, 72.83; H, 8.05.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3,17-Diacetate 21-Benzoate (XVd).—A suspension of 2 g. of the 3-acetate 21-benzoate XVc in 100 cc. of acetic anhydride was stirred at room temperature with 1.1 g. of *p*-toluenesulfonic acid hydrate for 65 hours. Pouring into water, followed by collection and crystallization of the resulting precipitate from chloroform–methanol produced 2.06 g. (95%) of the triester XVd with m.p. 205–209°. A further purified sample showed m.p. 208–210°.

Anal. Calcd. for $C_{32}H_{40}O_7$: C, 71.61; H, 7.51. Found: C, 71.88; H, 7.86.

Δ^5-Pregnene-3β,17α,21-triol-20-one 3-Acetate 21-Phenyl Ether (XVe).—The total iodo compound XIV obtained by bromination and sodium iodide displacement of 15 g. of XIIIa (see preparation of XVa, above) was dissolved in 100 cc. of dry acetone and added to a mixture of 15 g. of potassium bicarbonate and 60 g. of phenol in 450 cc. of acetone, which had previously been boiled for 1 hour. The suspension was boiled under reflux for 15 hours, most of the solvent was removed and the residue was diluted with water. Crystallization of the precipitate from acetone furnished 9.25 g. (50% from XIIIa) of the 21-phenyl ether XVe with m.p. 228–230°, $[\alpha]_D$ −6°.

Anal. Calcd. for $C_{29}H_{38}O_5$: C, 74.65; H, 8.21. Found: C, 75.00; H, 8.40.

Δ^5-Pregnene-3β,7α,21-triol-20-one 3,17-Diacetate 21-Phenyl Ether (XVf).—The C-17 acetylation of 5 g. of XVe was carried out as usual with 2.8 g. of *p*-toluenesulfonic acid hydrate in 300 cc. of acetic anhydride for 24 hours at room temperature. Dilution with water and crystallization from chloroform–methanol yielded 4.1 g. (75%) of XVf with m.p. 168–170°, $[\alpha]_D$ −58°.

Anal. Calcd. for $C_{31}H_{40}O_6$: C, 73.20; H, 7.93. Found: C, 72.88; H, 8.16.

Δ^5-Pregnene-3β,17α,21-triol-20-one 17-Acetate 21-Phenyl Ether (XVg).—A solution of 5 g. of XVf in 900 cc. of methanol was cooled to 10° and 5 g. of potassium hydroxide in 100 cc. of methanol, also cooled to 10°, was added. After being allowed to stand at 10° for 90 minutes under nitrogen, the solution was neutralized with acetic acid, most of the solvent was evaporated and the residue was diluted with water. The precipitate (4.54 g., m.p. 110–119°) was crystallized from benzene and yielded 0.68 g. (15%) of the 3-acetate 21-phenyl ether XVe with m.p. 222–226°, undepressed on admixture with a sample of this substance (m.p. 228–230°) described above.

The benzene mother liquors were chromatographed on 400 g. of neutral alumina. The fractions eluted with benzene furnished 0.36 g. (7%) of starting material XVf with m.p. 165–168° (undepressed on admixture with XVf) while the fractions eluted with benzene–ether (4:1) on crystallization from acetone–hexane produced 2.25 g. (49%) of the 17-acetate 21-phenyl ether XVg with m.p. 161–165°. The analytical sample exhibited m.p. 166–168°, $[\alpha]_D$ −50°. A large depression in m.p. was observed on admixture with the starting material XVf.

Anal. Calcd. for $C_{29}H_{38}O_5$: C, 74.65; H, 8.21. Found: C, 74.32; H, 8.03.

Δ^4-Pregnene-17α,21-diol-3,20-dione (Substance S) 17-Acetate 21-Phenyl Ether (XVI).—Aluminum isopropoxide (1.1 g.) was added to a solution of 2.2 g. of the 17-acetate 21-phenyl ether XVg in 90 cc. of dry toluene and 30 cc. of cyclohexanone and the mixture was boiled under reflux for 1 hour. The volatile components were then removed by steam distillation, the solid was collected and well extracted with hot acetone. Evaporation of the extract, followed by crystallization of the residue from acetone–pentane, led to 1.78 g. (81%) of substance S 17-acetate 21-phenyl ether (XVI) with m.p. 146–150°. A further purified specimen showed m.p. 149–151°, $[\alpha]_D$ +49°, λ_{max} 240 and 276 mμ, log ϵ 4.25 and 3.20, respectively.

Anal. Calcd. for $C_{29}H_{36}O_5$: C, 74.97; H, 7.81. Found: C, 74.73; H, 7.62.

Attempts to remove the phenyl ether function under a variety of conditions, both acidic and alkaline, either left this group intact or resulted in decomposition of the side chain.

MEXICO D. F., MEXICO

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA, LOS ANGELES]

Anthochlor Pigments. XI. The Constituents of *Coreopsis maritima*. Reinvestigation of *Coreopsis gigantea*

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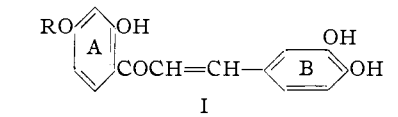
By means of paper chromatography and ultraviolet spectroscopy, the constituents of the flowers of *Coreopsis maritima* have been identified as butein, coreopsin, marein, maritimein, luteolin-7-glucoside and sulfurein. Reinvestigation of *C. gigantea*, a plant morphologically similar to *C. maritima*, has shown the presence in the flowers of all of the same pigments, except that the luteolin glucoside appears to be absent. The biogenetic significance of the pigmentation of these two species of the genus *Coreopsis* is discussed.

The anthochlor pigments of species of the genus *Coreopsis* have been the subject of investigations in these laboratories[1] and elsewhere.[2] In this earlier work, the pigments, mainly chalcone and aurone glycosides, were isolated by classical methods and no attempt was made to identify all the pigments occurring in a single species. Recently, flowers of *Coreopsis maritima*, a hitherto uninvestigated species, became available, and an examination of all the major constituents was undertaken with the use of a combination of paper chromatography and ultraviolet spectroscopy. As a part of an investigation of flavonoid biogenesis, the main object was to study all of the pigments present in one plant in order to gain more detailed information on their interrelationships.

A fresh, concentrated extract of *C. maritima* flowers was separated by chromatography on thick paper into five main bands. These bands were then eluted separately with aqueous ethanol, and purified by rechromatography. The spectral characteristics and R_f values of the purified eluates were then studied. Comparison of these spectral results, combined with the R_f values in several different solvent systems, with measurements on known compounds served to identify the majority of the constituents. The compounds identified were butein (I, R = H), chlorogenic acid (II), coreopsin [I, R = Gl (glucosyl)], sulfurein (III, R = Gl), luteolin 7-glucoside (IV), marein (V, R = Gl) and maritimein (VI, R = Gl).

Butein (I, R = H) and its 4′-glucoside, coreopsin, have been isolated from several other *Coreopsis* species[1,2] and together constitute perhaps the most commonly occurring of all anthochlor pigments. The corresponding aurone, sulfurein (III, R = Gl) previously has been found in *Cosmos sulfureus*[2,3] and as the aglucone, sulfuretin, in yellow Dahlia.[4] The presence of chlorogenic acid (II) has not previously been detected in the genus *Coreopsis*, but it occurs in all members of this species that have



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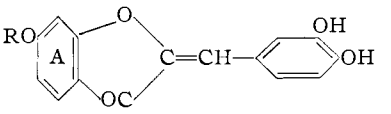

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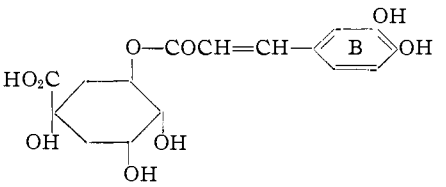

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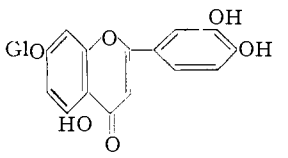

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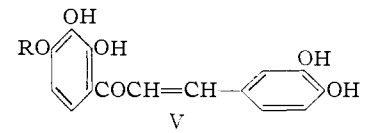

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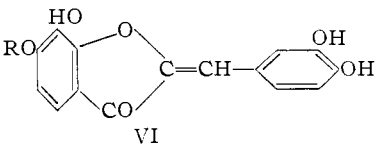

VI

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