17,21-Dihydroxy-5β-pregn-2-ene-1,11,20-trione, a New Analogue of Cortisone. Methods for Distinguishing Between Δ^2-1-Oxo- and Δ^1-3-Oxo-steroids

By **John J. Schneider,** Department of Medicine, Jefferson Medical College, Philadelphia, Pennsylvania 19107, U.S.A.

17,21-Dihydroxy-5β-pregn-2-ene-1,11,20-trione (9), a new analogue of cortisone, has been prepared from 1β,3α,17,20β,21-pentahydroxy-5β-pregnan-11-one, and compared with known analogues, namely 'isocortisone' (11) and 'Δ^1-allocortisone' (2). The chief characteristic of the new analogue is its instability, which is attributed to interaction between the C-1 and C-11 carbonyl groups with resulting conformational distortion.

A study has been made of the u.v. and i.r. absorption and rotational characteristics of eleven matched pairs of Δ^2-1-oxo- Δ^1-3-oxo-steroids in order to better characterise and differentiate them. Significant and consistent differences were noted in their extinction coefficients, in the C=C stretching bands of their i.r. spectra, and in their M_D values. Examples of C-1 carbonyl:C-10 methyl and C-1 carbonyl:C-11 carbonyl group interactions, derived from M_D considerations, also were encountered.

PREVIOUSLY prepared analogues of cortisone (1) include 'Δ^1-allocortisone' (17,21-dihydroxy-5α-pregn-1-ene-3,11,20-trione) (2),[1] in which the double bond is transposed from the 3,4- to the 1,2-position, and 'isocortisone' (17,21-dihydroxy-5β-pregn-1-ene-3,11,20-trione) (11),[2] which differs from (2) in that rings A and B are *cis*- rather than *trans*-fused. We now describe the synthesis and some of the properties of a third analogue, namely 17,21-dihydroxy-5β-pregn-2-ene-1,11,20-trione (9), the structure of which incorporates transposition of the double bond to the 2,3-position, shifting of the C-3 carbonyl group to C-1, and a *cis*-fused AB ring system.

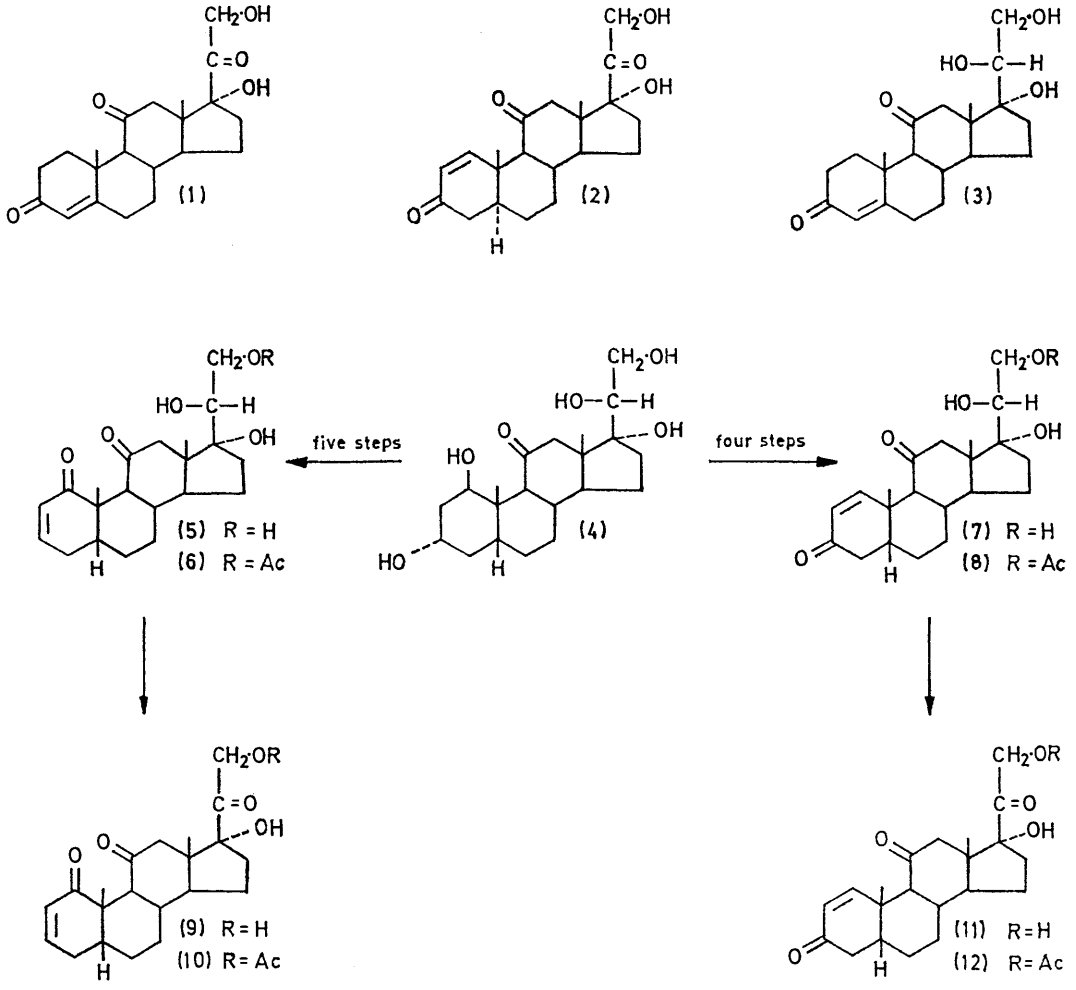
[1] E. Wilson and M. Tishler, *J. Amer. Chem. Soc.*, 1952, **74**, 1609.

[2] V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, 1951, **188**, 287.

The new analogue was prepared, and an alternative route to 'isocortisone' was devised, by extending reaction sequences developed earlier.[3,4] In outline, 1β,3α,17,20β,21-pentahydroxy-5β-pregnan-11-one (4) was converted into its 20,21-isopropylidene derivative and oxidised with chromic anhydride in pyridine. Chromatography of the neutral reaction mixture provided chiefly the 3-hydroxy-1-ketone, but also the 1-hydroxy-3-ketone. Treatment of the former with acetic anhydride in pyridine, followed by percolation of the acetate through neutral alumina and removal of the acetonide group, gave the free glycerol (5). The 1-hydroxy-3-ketone was not utilised at the time, but it was observed in another series [3] that a 1,2-double bond could be introduced readily by a base-catalysed dehydration. Application of this reaction in the present instance, followed by removal of the acetonide group, gave the free glycerol (7).

The remaining important step in each synthesis consisted of oxidation of the glycerol type of side chain to the 17,21-dihydroxy-20-oxo-system. As illustrated by Sarett's first synthesis of cortisone,[5] the procedure consists of selective acetylation at C-21 (which can be effected in reasonable yield), followed by oxidation with chromic anhydride under relatively mild conditions (which is so handicapped by cleavage of the 17,20-bond as to afford the desired ketol acetate in less than 25% yield).

Sarett prepared the 21-monoacetate of the glycerol (3) in 87% yield by treating it with an excess of acetic anhydride in pyridine, chromatographing on alumina, and re-cycling the non-crystalline fractions. We modified this method to the extent of using a slight excess of the anhydride (in pyridine) for 24 h at room temperature, followed by chromatography on a single, more selective (partition-type) column. The 21-monoacetate band was easily identified by paper chromatography but otherwise not formally characterised. Treatment of the glycerol (7) in this fashion furnished its 21-monoacetate (8) in 72% yield, but a distinctly lower yield of the 21-monoacetate (6) (50%) was obtained from the glycerol (5).



[3] J. J. Schneider and N. S. Bhacca, *J. Biol. Chem.*, 1966, **241**, 5313.

[4] J. J. Schneider, P. Crabbe, and N. S. Bhacca, *J. Org. Chem.*, 1968, **33**, 3118.

[5] L. H. Sarett, *J. Biol. Chem.*, 1946, **162**, 601.

In effecting oxidation of the glycerol 21-monoacetates to the respective 20-oxo-derivatives, we employed the modification of Walker,[6] who found that in the oxidation of three 11-oxygenated 17,20α,21-trihydroxy-21-acetates the addition of manganous salts (to an otherwise standard chromic anhydride system) raised the yield of the 20-oxo-21-acetates from an average of 24% to an average of 50%. We observed a similar effect in the oxidation of the glycerol 21-monoacetate (8), which furnished the 20-oxo-21-acetate (12) in 45% yield. But similar oxidation of the glycerol 21-monoacetate (6) afforded the desired 20-oxo-21-acetate (10) in only 30% yield. Both ketol acetates were hydrolysed to their respective 21-ols by the transesterification method.[7]

These studies provided six pairs of Δ^2-1-oxo- and Δ^1-3-oxo-steroids, all AB-*cis*. Since relatively few such steroids have been prepared to date, and since their structural diversity makes comparisons difficult, we examined these pairs in a variety of ways, both in order better to characterise each type of ketone as a class, and to determine to what extent the two types can be differentiated. The Table gives the sources of these compounds together with their $\lambda_{max.}$ values and extinction coefficients, their C=C stretching frequencies, and their M_D values. It also gives the same constants for a series of enol methyl ethers prepared earlier (see Table II of ref. 4); these bear the same conjugated unsaturated systems and thus logically extend the series.

Paired Δ^2-1-oxo and Δ^1-3-oxo-steroids; comparisons of u.v., i.r., and rotational data

Stem compound	Conj. unsat. system	No. or ref.*	$\lambda_{max.}$/nm	ε	$\nu_{max.}$/cm^{-1} (C=C str.)	M_D	$M_D(\Delta^1) - M_D(\Delta^2)$
5β-Androstan-17-one	Δ^2-1-Oxo	Ref. 3	225	7650	1621	+9	+671
	Δ^1-3-Oxo	Ref. 3	230	9600	1600	+680	
5β-Androstane-11,17-dione	Δ^2-1-Oxo	Ref. 3	225	7100	1620	+283	+375
	Δ^1-3-Oxo	Ref. 3	224	9425	1600	+658	
17-Hydroxy-5β-pregnan-11-one-20,21-acetonide	Δ^2-1-Oxo	Ref. 4	225	7450	1628	+12	+448
	Δ^1-3-Oxo	Unpub.	225	9061	1602	+460	
17,20β,21-Trihydroxy-5β-pregnan-11-one	Δ^2-1-Oxo	(5)	225	7410	1630	+54	+388
	Δ^1-3-Oxo	(7)	225	9300	1601	+442	
17,21-Dihydroxy-5β-pregnane-11,20-dione acetate	Δ^2-1-Oxo	(10)	225	6500	1628	+326	+374
	Δ^1-3-Oxo	(12)	224	8700	1603	+704	
17,21-Dihydroxy-5β-pregnane-11,20-dione	Δ^2-1-Oxo	(9)	224	7064	1629	+152	+381
	Δ^1-3-Oxo	(11)	223	9500	1604	+533	
1 (or 3)-Methoxy-5β-androstane-11,17-dione	Δ^2-1-Oxo	(13)	250	12,430	1619	+92	+446
	Δ^1-3-Oxo	(14)	258	14,850	1582	+538	
1 (or 3)-Methoxy-5β-androstan-17-one	Δ^2-1-Oxo	(15)	250	14,700	1619	−155	+740
	Δ^1-3-Oxo	(16)	254	16,300	1590	+595	
1 (or 3)-Methoxy-5α-androstan-17-one	Δ^2-1-Oxo	(17)	248	13,030	1616	+879	−481
	Δ^1-3-Oxo	(18)	255	15,400	1570	+398	
(25*S*)-1 (or 3)-Methoxy-5α-spirostane	Δ^2-1-Oxo	(19)	248	14,670	1615	+234	−552
	Δ^1-3-Oxo	(20)	256	16,770	1575	−318	
1 (or 3)-Methoxy-5α-cholestane	Δ^2-1-Oxo	(21)	237	14,500	1618	+601	−444
	Δ^1-3-Oxo	(22)	242	15,850	1581	+157	

* Full names of compounds (13)—(22) are given in Table II of ref. 4.

The structures of the ketol (11) and its acetate (12) were confirmed by direct comparisons with authentic specimens of 'isocortisone' and its acetate. The constitution of the new ketol (9) follows from its mode of synthesis and the observation that oxidation of the glycerol (5) with periodic acid furnished the known 5β-androst-2-ene-1,11,17-trione.[3]

The low yields encountered in the preparation and subsequent oxidation of the 21-monoacetate (6), and the deterioration of the free ketol (9) (which turned brown within a few weeks), indicate an inherent instability of Δ^2-1,11-diketones. We attribute this to interaction between the carbonyl groups at C-1 and C-11 with resulting conformational distortion. Other examples of the effects of interactions in steroids bearing oxygen functions at both C-1 and C-11 are given in our earlier papers.[3,4,8]

† A recent compilation [9] lists sixteen Δ^1-3-ketones [average $\lambda_{max.}$ 231 nm (range 222—250 nm), and average ε 9920 (range 6100—17,378)], and five Δ^2-1-ketones [average $\lambda_{max.}$ 224 nm (range 220—226 nm), and average ε 8085 (range 7762—8511)].

‡ This closer relationship in the case of Δ^1-3-ketones may account for the fact that they are far more sensitive (as reflected in their u.v. spectral characteristics) to the introduction of additional groups than are Δ^2-1-ketones (note the marked differences in ranges in the two types in ref. 9).

In discussion I will refer to the first series as group A and the second as group B.

In the case of group A, the u.v. absorption peaks in both series are too similar (at or near 225 nm for most members) to permit differentiation, but the extinction coefficients of the Δ^2-1-ketones (average 7200) are significantly lower, individually and generally, than those of the Δ^1-3-ketones (average 9200).† The $\lambda_{max.}$ values agree well with that predicted from the Woodward–Fieser approximations [227 nm (215 plus 12 nm for one β alkyl residue)] but it is more difficult to account for the regular differences in extinction coefficients. The lower coefficients in the Δ^2-1-ketones may reflect the fact that the double bond is more distant from a highly substituted carbon atom (βγ to either C-5 or C-10) whereas the bond in Δ^1-3-ketones is αβ to, and thus more influenced by, the tetrasubstituted C-10 site.‡ Within the limits imposed by this small

[6] B. H. Walker, *J. Org. Chem.*, 1967, **32**, 1098.
[7] Huang-Minlon, E. Wilson, and M. Tishler, *J. Amer. Chem. Soc.*, 1952, **74**, 5394.
[8] J. J. Schneider, *J. Chromatog.*, 1968, **37**, 89.
[9] J. P. Dusza, M. Heller, and S. Bernstein, in 'Physical Properties of the Steroid Hormones,' ed. L. L. Engel, Macmillan, New York, 1963, pp. 132—134.

series, the coefficients are remarkably uniform within each series. This suggests (*a*) that they are largely uninfluenced by groups elsewhere in the molecule or (*b*) that, with the possible exception of the C-11 carbonyl group, the additional potential auxochromes are too distant from the conjugated unsaturated system to exert an effect.

In the case of group B, the Δ^2-1-ketones show u.v. maxima at a slightly, but consistently, lower wavelength (average 247 nm) than the Δ^1-3-ketones (average 253 nm). The first of these values is distinctly lower than, and the second close to, the predicted approximation [257 nm (215 plus 12 nm for one β-alkyl residue and 30 nm for one β-methoxy-residue)].[10] Since these differences are not shown by the pairs in group A, the *low* values for the Δ^2-1-oxo-ethers represent significant, but unaccounted for, deviations. (It is equally difficult to account for the low values given by *both* members of the cholestane pair, particularly since their remaining constants are in close accord with the others.) Second, the extinction coefficients qualitatively parallel the $\lambda_{max.}$ values and display a difference, within pairs, remarkably close to the values provided by group A. Thus the average extinction coefficient in the Δ^2-1-ketone series is 13,870 and that for the Δ^1-3-ketones is 15,830; the average difference within pairs is 1970. Since the nature and site of a given auxochrome and the observed *intensity* of absorption of a given chromophore cannot as yet be related in numerical terms, we can only record that, by appropriately comparing the first two pairs of each group (which have the same stem compounds), the effect of the methoxy-group is to increase the extinction coefficient, on average, by 83% in the case of the Δ^2-1-ketones and by 63% in the case of the Δ^1-3-ketones.

For the group A members, the C=C stretching band of the Δ^2-1-ketones occurs at a consistently higher frequency (range 1620—1630 cm^{-1}) than in the case of the Δ^1-3-ketones (1600—1604 cm^{-1}). This band was clearly visible in spite of its low intensity and its proximity, in some instances, to wide bands in the carbonyl region. These data confirm the observations of Jones *et al.*,[11] who noted this band in five Δ^1-3-ketones of varying structure (range 1604—1609 cm^{-1}), and supplement the value (1628 cm^{-1}) recorded for a Δ^2-1-ketone of unstated structure in the atlas of Roberts *et al.*[12]

The corresponding bands for the $CH_3{\cdot}O{-}C{=}C{-}C{=}O$ systems of the ethers of group B showed a similar trend: Δ^2-1-ketones absorb at a wavelength (average 1617 cm^{-1}) consistently higher than that displayed by the Δ^1-3-ketones (1579 cm^{-1}). This method allows one readily to distinguish between Δ^2-1-ketones and Δ^1-3-ketones but its general utility will depend on the band frequency, within the limits of 1600 and 1630 cm^{-1}, characteristic of other αβ-unsaturated ketones.

For the compounds in group A, M_D values of the Δ^2-1-ketones (average +140; range +9 to +326) are significantly smaller, individually and generally, than those of Δ^1-3-ketones (average +584; range +442 to +704). These uniformly large differences [expressed as $M_D(\Delta^1) - M_D(\Delta^2)$] show that this approach offers an assured means of distinguishing between these pairs of unsaturated ketones. Usually such large effects are attributed to conformational differences. But this is not apparent in Dreiding models of a representative pair; the shape of ring A and its spatial relationship to the remaining ring system are similar in both types. However the carbonyl group in the Δ^2-1-ketone lies sufficiently close to the methyl group at C-10 to suggest a degree of steric hindrance or shielding.

The M_D values for the ethers in group B served both to confirm and to qualify the above results. The pairs (13),(14) and (15),(16) (both AB-*cis*) gave differences which were positive, and of the same general magnitude, as the pairs from group A. But the differences provided by the three remaining pairs [(17),(18); (19),(20); and (21),(22) (all AB-*trans*)] are uniformly negative, and of the same magnitude. The (15),(16) pair (AB-*cis*) and the (17),(18) pair (AB-*trans*) are otherwise identical, and Dreiding models offer a logical explanation for the observed reversal in sign. The effect of shifting from AB-*cis* to *trans* in Δ^1-3-ketones is negligible beyond making the molecule more planar. But in the Δ^2-1-ketone series the change shifts the position of the C-1 carbonyl group from one approaching the C-10 methyl group (already noted) to one where it projects toward the α (rear) surface of the molecule and away from the methyl group. Thus a C-1 ketone : C-10 methyl group interaction could well occur in AB-cis-Δ^2-1-ketones, and this could account for the positivity of the observed differences. The *magnitude* of the differences in both the AB-*cis* and *trans* pairs must be attributed to other factors; these do not include the effect of substituting one configuration for another at C-5 since this is negligibly small.

The M_D data also show the marked effect of a C-11 carbonyl function in both groups on the M_D value of Δ^2-1-oxo-compounds (ΔM_D +274 in group A and +247 in group B) compared with the negligible effect in Δ^1-3-oxo-compounds (ΔM_D −22 in group A and −57 in group B).† This clearly indicates a C-1 : C-11 carbonyl group interaction.

The regularity of the differences and the general clarity of the results in the Table are due presumably to the fact that we are dealing with pairs of steroids the members of which differ only with respect to the location of the conjugated unsaturated system. In view of the variable, and largely unpredictable, effects of additional groups, particularly those near the double bond, on (for example) u.v. spectra, we doubt that these observations can be developed into a general method suitable for the

† I thank a referee for pointing this out.

[10] A. I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Products,' Macmillan, New York, 1964, p. 58.

[11] R. N. Jones, P. Humphries, E. Packard, and K. Dobriner, *J. Amer. Chem. Soc.*, 1950, **72**, 86.

[12] G. Roberts, B. S. Gallagher, and R. N. Jones, 'Infrared Absorption Spectra of Steroids,' vol. II, Interscience, New York, 1958, p. 27.

characterisation of unmatched pairs or individual compounds. We believe that, of the three approaches, the i.r. method will prove least subject to such perturbations.

The relative physiological activities of the three cortisone analogues can, to a degree, be correlated with their structures. ' Δ^1-Allocortisone ' retains some cortisone-like activity; it shares the *trans*-fused (planar) AB ring system with cortisone, and differs from it only with respect to the position of the double bond. In contrast, ' isocortisone ' is wholly inactive; in addition to the relocation of the double bond, *cis*-fusion of the AB ring system serves sharply to deflect ring A towards the enzymically important α (rear) surface of the molecule. The new analogue has not been obtained in amounts sufficient to determine its biological activity, but it too would be expected to be inactive, for it includes a degree of conformational distortion in addition to the *cis*-fused AB ring system.

A second type of structure–activity correlation was observed when we attempted to oxidise the glycerols (3), (5), and (7) to the corresponding 17,21-dihydroxy-20-ketones by use of the readily available 20β-hydroxysteroid dehydrogenase in the presence of β-diphosphopyridine nucleotide (β-DPN) at pH 8—9.[13] Unfortunately, the rate was impractically slow for substrates bearing several oxygen substituents, and was influenced also by the site of such functions. However, under conditions judged optimal, the glycerol (7) was oxidised at about one-third the rate observed for (3), and the glycerol (5) was not oxidised at all. These results may be understood, in a general way, in terms of the conformational factors already indicated.

EXPERIMENTAL

General experimental and analytical methods are described in refs. 3 and 4. In the present work all rotations were determined for methanolic solutions unless otherwise indicated. I.r. spectra were obtained for KBr dispersions with a Beckman IR-8 instrument. The following chromatographic systems were used (proportions by volume): (1) t, 110; iso, 90; M, 160; W, 40; (2), t, 140; iso, 60; M, 160; W, 40; (3) t, 70; iso, 130; M, 160; W, 40; (4) t, 120; iso, 80; M, 160; W, 40; (5) t, 200; M, 150; W, 50 (t = toluene; EA = ethyl acetate; M = methanol; W = water; iso = iso-octane).

17,20β,21-*Trihydroxy*-5β-*pregn*-1-*ene*-3,11-*dione* (7).—A solution of 1β,17-dihydroxy-20,21-isopropylidenedioxy-5β-pregnane-3,11-dione, m.p. 236—238°, $[\alpha]_D$ +25°,[4] (180 mg) in methanol (15 ml) and N-sodium hydroxide (10 ml) was kept at room temperature for 3 h. The neutral product was recovered and the acetonide group was removed;[4] and the free glycerol crystallised from aqueous methanol as *needles* (110 mg), m.p. 132—133 and 186—187°; $[\alpha]_D$ +122°; $\nu_{max.}$ 3560—3200 (OH), 1690 (C-11 CO), and 1665 and 1601 cm^{-1} (Δ^1-3-ketone) (Found: C, 69·4; H, 8·2. $C_{21}H_{30}O_5$ requires C, 69·6; H, 8·35%).

21-*Acetoxy*-17,20β-*dihydroxy*-5β-*pregn*-1-*ene*-3,11-*dione* (8).—To a solution of compound (7) (200 mg, 0·55 mmol) in pyridine (2 ml), acetic anhydride (0·076 ml, 0·75 mmol) was added. After 24 h at room temperature, the neutral fraction was recovered and chromatographed on a 25 × 750 mm Celite column prepared and developed with system (1). The yield of 21-monoacetate (8) was 155 mg (72%).

21-*Acetoxy*-17,20β-*dihydroxy*-5β-*pregn*-3-*ene*-1,11-*dione* (6).—17,20β,21-Trihydroxy-5β-pregn-2-ene-1,11-dione (5) [m.p. 281·5—283°; $[\alpha]_D$ +15°; $\nu_{max.}$ 3570—3220 (OH), 1700 (C-11 CO), and 1668 and 1630 cm^{-1} (Δ^2-1-ketone)] (200 mg) was acetylated and chromatographed [system (2)] as in the preparation of (8). The yield of chromatographically homogeneous 21-monoacetate (6) was 110 mg (50%).

21-*Acetoxy*-17-*hydroxy*-5β-*pregn*-1-*ene*-3,11,20-*trione* (12).—Oxidation of the dione (8) (125 mg) by the method of Walker,[6] followed by chromatography on a 25 × 800 mm Celite column [system (3)] and crystallisation of the product from methanol–acetone, gave needles (56 mg, 45%), m.p. 242—243°; $[\alpha]_D$ +175°; $\nu_{max.}$ 3520—3300 (OH), 1750—1705 (C-11 and C-20 CO), and 1660 and 1603 cm^{-1} (Δ^1-3-ketone) {lit.,[2] m.p. 245—246°; $[\alpha]_D$ +138° (in Me_2CO)} (Found: C, 68·5; H, 7·4. Calc. for $C_{23}H_{30}O_6$: C, 68·65; H, 7·5%).

21-*Acetoxy*-17-*hydroxy*-5β-*pregn*-2-*ene*-1,11,20-*trione* (10).—Similar oxidation of the dione (6) (125 mg), followed by chromatography on a 25 × 760 mm Celite column [system (4)] gave, from acetone–n-hexane, *needles* (38 mg, 30%), m.p. 252—253°; $[\alpha]_D$ +81°; $\nu_{max.}$ 3550—3295 (OH), 1750—1705 (C-11 and C-20 CO), and 1675 and 1628 cm^{-1} (Δ^2-1-ketone) (Found: C, 68·4; H, 7·7. $C_{23}H_{30}O_6$ requires C, 68·65; H, 7·5%).

17,21-*Dihydroxy*-5β-*pregn*-1-*ene*-3,11,20-*trione* (11).—To a solution of the acetate (12) (50 mg) in dry methanol (10 ml) under dry nitrogen, a solution of sodium methoxide (*ca.* 1 mg) in dry methanol (1 ml) was added, with stirring. After 10 min at room temperature, an excess of acetic acid was introduced, the mixture was evaporated *in vacuo*, and the residue was chromatographed on a 16 × 600 mm Celite column [system (2)]. The recovered ketol (42 mg) crystallised from ethyl acetate as *needles*, m.p. 212·5—213·5°; $[\alpha]_D$ +170°; $\nu_{max.}$ 3550—3210 (OH), 1725—1705 (C-11 and C-20 CO), and 1665 and 1604 cm^{-1} (Δ^1-3-ketone) (Found: C, 69·75; H, 7·9. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%).

17,21-*Dihydroxy*-5β-*pregn*-2-*ene*-1,11,20-*trione* (9).—Similar hydrolysis of the acetate (10) (25 mg) and chromatography on a 12 × 600 Celite column [system (5)] gave material (20 mg) which crystallised from acetone–n-hexane as *prisms*, m.p. 240·5—241·5°; $[\alpha]_D$ +58°; $\nu_{max.}$ 3550—3220 (OH), 1720—1705 (C-11 and C-20 CO), and 1665 and 1629 cm^{-1} (Δ^2-1-ketone) (Found: C, 69·7; H, 7·75. $C_{21}H_{28}O_5$ requires C, 70·0; H, 7·8%).

This work was supported by a grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service. I thank Dr. V. Mattox for a reference sample of ' isocortisone ' acetate, and Dr. E. P. Vollmer for a report on its biological activity. Dr. S. Bernstein kindly pointed out the Scott monograph to me.

[3/201 *Received*, 29*th January*, 1973]

[13] P. Talalay, in ' The Enzymes,' vol. 7, ed. P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, 1963, p. 177.