(II) and 0.3462 g (19.16 mmoles) of ^{18}O-enriched water (1.40 atom % ^{18}O) was shaken until the mixture was homogeneous and then allowed to stand at room temperature for 190 min. The solution was taken up in ethyl ether and passed through a column of Linde Type 3-A Molecular Sieve (predried at 500°F) to remove water and ethanol. Removal of ether and evaporative distillation [*ca.* 50° (8 mm)] provided the sample of 2-cyclohexenone, which was shown by gas chromatographic analysis to contain 2.3 ± 0.4% unreacted II, indicating that the reaction had proceeded to 98% completion. A 17-mg sample of the unsaturated ketone was pyrolyzed at 600–650° in an evacuated Vycor tube, and the carbon monoxide was analyzed mass spectrometrically. A mixture of 0.5202 g (5.42 mmoles) of 2-cyclohexenone and 0.3485 g (19.4 mmoles) of ^{18}O-enriched water was subjected to identical treatment as a "control" experiment. A sample of pure 2-cyclohexenone was also pyrolyzed to provide carbon monoxide of "normal abundance." The atom per cent of ^{18}O of each sample was determined from the ratio of the peaks at *m/e* 28 and 30. The results are in Table II.

B. Examination of Ethanol.—A mixture of 0.7026 g (4.95 mmoles) of 3-ethoxy-2-cyclohexenol (II) and 0.3750 g (20.8 mmoles) of ^{18}O-enriched water was allowed to react as described under A. The ethanol produced was separated from water and cyclohexenone by gas chromatography using a 7.5 ft × $^3/_8$ in. column of triethylene glycol on 30–60 Chromosorb P (30 g of absorbent/100 g of support) at 110°. The ethanol was collected from the chromatogram and analyzed directly in the mass spectrometer. A "control" sample was prepared by treating 0.4656 g (10.1 mmoles) of ethanol and 0.7174 g (39.9 mmoles) of ^{18}O-enriched water under the same conditions. A sample of II was hydrolyzed in ordinary water to give the ethanol which served to indicate "normal" ^{18}O content. The mass spectrum of the three samples was the same within the experimental error, as the following relative intensities show (*m/e* (31) = 100) (see Table IV).

TABLE IV

m/e	Normal	Control	Reaction
46	15.9	15.9	15.9
47	0.52	0.49	0.49
48	0.048	0.048	0.044

Calculations.—The hydrolysis of the alkoxyallylic alcohol in a quantity of ^{18}O-enriched water, sufficient to maintain essentially a constant level of ^{18}O enrichment in the solvent reservoir, would produce cyclohexenone containing this same level of enrichment, since the ketonic oxygen is derived from the water. In the experiments reported here, however, the substrate and the labeled water were taken in comparable amounts, so that the ^{18}O content of the water changed during the reaction, being diluted by oxygen from the substrate. In order to calculate the ^{18}O content to be expected in the cyclohenone product one has to differentiate between two kinetic situations in the mechanism of eq 3–7. If $k_{-4} \gg k_5$ (case I) mixing will occur between the alcohol oxygen of the substrate and water prior to the formation of product. The ^{18}O content of the cyclohexenone would then be simply the average of all the labile oxygens. If, on the other hand $k_5 \gg k_{-4}$ (case II), the ^{18}O content of the cyclohexenone (and of the water) would change continuously as the reaction proceeds. The level of ^{18}O labeling in the product can be predicted on the basis of eq iii derived below.[16] It is clear that an intermediate kinetic situation ($k_{-4} \sim k_5$) would lead to values intermediate between those calculated for case I and case II.

For the reaction

$$\mathrm{II} + \mathrm{H_2O} \longrightarrow \mathrm{III} + \mathrm{H_2O} + \mathrm{EtOH}$$

$$dW^{18} = -d[II]\left\{\frac{[II^{18}]W^{16}}{[II]W} - \frac{[II^{16}]W^{18}}{[II]W}\right\} \quad \text{(i)}$$

where W^{18} refers to $[H_2{}^{18}O]$, W to total water, etc. Let $\alpha = W^{18}/W$ (a variable), $\beta = [II^{18}]/[II]$ (a constant, neglecting kinetic isotope effects), and x = a reaction variable, so that $-d[II] = [II]_0 dx$. Then

$$\frac{d\alpha}{\beta - \alpha} = \frac{[II]}{W}dx \quad \text{(ii)}$$

Integration of eq ii gives

$$\alpha = \beta + (\alpha_0 - \beta)e^{-[II]_0 x/W} \quad \text{(iii)}$$

Experimentally, $[II]_0 = 4.98$, $W = 19.16$, $\alpha_0 = 0.0140$, $\beta = 0.00242$, $x = 0.977$, whence $\alpha = 0.0114$ after 97.7% reaction. From the stoichiometry one calculates a corresponding value of 1.3 atom % for the cyclohexenone.

Registry No.—II, 7650-73-9; carbon monoxide, 630-08-0; I, 5323-87-5; III, 930-68-7.

Acknowledgment.—We are indebted to Mr. Frank Drogosz for operation of the mass spectrometer.

(16) We are grateful to Professor C. E. Nordman for advice which greatly simplified the derivation of eq iii.

The Effect of Manganese on the Chromic Acid Oxidation of Secondary–Tertiary Vicinal Glycols

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The chromic acid oxidation of the two steroidal secondary–tertiary vicinal glycols, "triol acetate" (1, 11β,17α,-20α,21-tetrahydroxy-4-pregnen-3-one 21-acetate) and "Δ¹-triol acetate" (2, 11β,17α,20α,21-tetrahydroxy-1,4-pregnadien-3-one 21-acetate), with or without added manganous ions, has been examined. In the absence of added manganous ions, glycol cleavage is the dominant reaction giving adrenosterone (androst-4-ene-3,11,17-trione, 11) and Δ¹-adrenosterone (androsta-1,4-diene-3,11,17-trione, 12), respectively. In the presence of manganous salts, oxidation of the secondary alcohol to ketone predominates over the cleavage reaction and cortisone acetate (9) and prednisone acetate (10) result. Some consideration is given to the mechanism of the reaction, the stereochemistry of the starting materials, and to the location of the acetate groups.

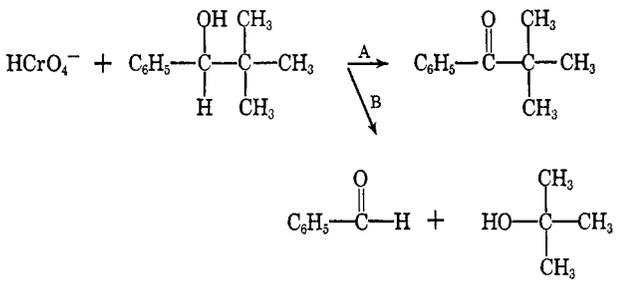
During the past 15 years a large number of papers have appeared which deal with the mechanism of chromic acid oxidation of alcohols.[1] Major contributions were made by Westheimer and his coworkers[2] who postulate the following scheme: $R_2CHOH + Cr^{6+} \rightarrow R_2C{=}O + Cr^{4+}$. In the initial step chromium(VI) is reduced by alcohol to chromium(IV). Chromium(IV) is an unstable state of chromium and in the presence of chromium(VI) decomposes as follows: $Cr^{4+} + Cr^{6+} \xrightarrow{\text{fast}} 2Cr^{5+}$. Chromium(V) is also unstable and rapidly reacts with alcohols to give

(1) For recent reviews, see (a) R. Stewart, "Oxidation Mechanisms," W. A. Benjamin, Inc., New York, N. Y., 1964, p 33; (b) K. B. Wiberg, "Oxidation in Organic Chemistry," Academic Press Inc., New York, N. Y., 1965, p 69.

(2) J. J. Cawley and F. H. Westheimer, *J. Am. Chem. Soc.*, **85**, 1771 (1963), and earlier papers.

ketone and the stable chromium(III): $2Cr^{5+} + 2R_2CHOH \xrightarrow{fast} 2R_2CO + 2Cr^{3+}$. The result of these reactions is that two-thirds of the ketone formed is generated not from chromium(VI) directly but indirectly from chromium(V).

The chromic acid oxidation of some alcohols results in cleavage in addition to oxidation. For example, phenyl-*t*-butylcarbinol[2,3] on oxidation with chromic acid proceeds by two routes. The extent of the



cleavage ranged from as much as 53% to as little as 3% depending on the exact conditions. By adding manganous or cerous ions to a reaction mixture, the amount of cleavage (path B) could be reduced markedly. Westheimer has concluded that the cleavage reaction most likely is caused by chromium(V) and in the presence of manganous or cerous ions this species is not present to a significant extent because the chromium(IV) needed to generate the -V species is reduced according to the following equations. One implication

$$Mn^{2+} + Cr^{4+} \longrightarrow Mn^{3+} + Cr^{3+}$$

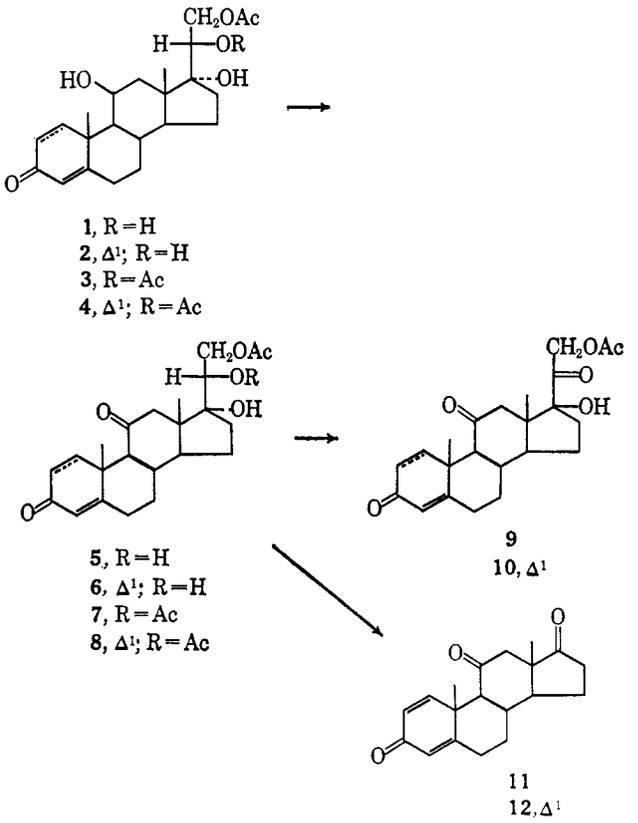
$$2Mn^{3+} \longrightarrow MnO_2 + Mn^{2+}$$

of this proposal is that in the oxidation of a secondary alcohol with chromic acid no more than 66.7% cleavage should occur if it is caused exclusively by chromium(V).

Chatterji,[4] Slack,[5] and Westheimer[6,7] have examined the oxidation of 1,2-glycols with chromic acid. From their work, it is apparent that chromic acid in acetic acid causes cleavage as well as oxidation. With the ditertiary glycols, *cis*- and *trans*-1,2-cyclopentanediol and pinacol, cleavage is essentially quantitative. The disecondary glycol, 2,3-butanediol, is cleaved to at least 30% and even ethylene glycol is cleaved on oxidation to the extent of at least 2%. It appears that increasing the alkyl substitution on an ethylene glycol increases the amount of cleavage. A reduction in the rate of oxidation in the presence of added manganese(II) and cerium(III) was noted in several of the above cases but the effect on the product distribution was not examined.

Results

Oxidation Studies.—We have found that both manganese(II) and cerium(III) markedly effect the product distribution in the chromic acid oxidation of **1** and **2**. The process outlined is the pathway for both the manganese-moderated and the unmoderated chromic acid oxidation. That oxidation occurs at C-11



first was shown by the following observations. Mild oxidizing conditions result in **5** being the major product. Very brief oxidation in the presence of manganese(II) also results in **5** being a dominant product. In addition, hydrocortisone (the product of oxidation at C-20 but not at C-11), has never been observed as a product.

The product distribution for the oxidation of **1**, **2**, and **5** is summarized in Table I. These data show the

TABLE I

PRODUCT DISTRIBUTION ON OXIDATION OF STEROIDAL GLYCOLS

Compd	Mn present	% cleavage	% oxidation	Total, %	Unaccounted for, %
1	Yes	21	50	71	29
1	No	74	14	88	12
5	Yes	14	48	62	38
5	No	56	30	86	14
2	Yes	22	56	78	26
2	No	57	28	85	15

marked effect of manganous nitrate on the ratio of oxidation to cleavage. In the presence of manganous salts, 1.5 to 3 times as much oxidation as cleavage occurs, while without manganous salts, cleavage dominates 2 to 1 over oxidation. The cerous nitrate moderated oxidation was investigated on one example only. In this case the yield of cortisone acetate (**4**) from **1** was found to be 42% compared to 14% in the unmoderated reaction. The 74% yield of cleavage product found (Table I) in the unmoderated oxidation of **1** contrasts with the theoretical yield of 66.7%. Since the products are stable under the reaction condition, we postulate that the excess cleavage is caused by the chromium(IV) and/or chromium(V) generated during the oxidation at C-11, causing additional side-chain cleavage. Consistent with this interpretation is the 56% yield of cleavage product found on oxidation of **5**, where the C-11 ketone was formed prior to the attack at C-20.

(3) J. Hampton, A. Leo, and F. H. Westheimer, *J. Am. Chem. Soc.*, **78**, 306 (1956).

(4) (a) A. C. Chatterji and S. K. Mukherjee, *Z. Physick. Chem.*, **207**, 372 (1957); (b) *ibid.*, **208**, 281 (1957); (c) *ibid.*, **210**, 166 (1959).

(5) R. Slack and W. A. Waters, *J. Chem. Soc.*, 597 (1949).

(6) J. Roček and F. H. Westheimer, *J. Am. Chem Soc.*, **84**, 2241 (1962).

(7) Y. W. Chang and F. H. Westheimer, *ibid.*, **82**, 1401 (1960).

TABLE II

NMR ABSORPTIONS OF STEROIDAL ALCOHOLS AND ACETATES. APPROXIMATE POSITION OF THE ABSORPTIONS OF THE 20 AND 21 HYDROGEN

Compd	Solvent	Center of C-20H absorptions, cps	Center C-21H absorptions, cps	δ_{20CH}[a]	δ_{21CH_2}[a]	$\delta\delta_{20OH}$[b]
13	d_6-DMSO d_7-DMF	213	213			
1	d_7-DMF	260–265	238	50	25	..
2	d_6-DMSO d_7-DMF	250–260	235	42	22	..
5	d_7-DMF	253	236	40	23	..
6	d_7-DMF	258–265	240	47	27	..
3	$CDCl_3$	321	258	108	45	58
4	$CDCl_3$	321	259	108	46	66
7	$CDCl_3$	317	253	104	40	64
8	$CDCl_3$	318	248	105	35	58

[a] δ refers to the shift of position of the absorption of the 20 and 21 hydrogens on acetylation relative to that of the reference alcohol. [b] $\delta\delta$ refers to the shift of the position of the absorption of the 20 hydrogen relative to its position in the corresponding monoacetate.

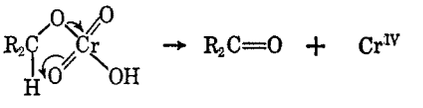
The yields reported are based on product isolation in most cases. For details, see the Experimental Section.

It is noteworthy that the percentage of unaccounted material (last column, Table I) is always higher when the oxidation is carried out in the presence of manganese(II). On the basis of chromatographic evidence, the materials in the unaccounted for category are highly polar and may well be acids generated mainly by cleavage of the 20,21 bond.

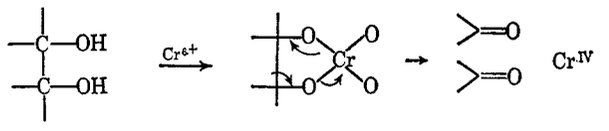
The selective oxidation of **1** to the corresponding 11-ketone (**5**) using either N-bromosuccinimide in pyridine or chromic acid in dimethylformamide is reported in the Experimental Section.

Stereochemistry.—The stereochemistry of the hydroxyls at 11, 17, and 20 in **1** was reported in earlier publications from these laboratories.[8,9] All of our data confirm the assignments made at the time; *i.e.*, the orientation of the hydroxyls is 11β,17α,20α. The stereochemical assignment for the Δ^1 analog (**2**) is based on its conversion to the known 20,21-diacetate (**4**),[10] its oxidation to the known 11-ketone (**6**),[11] and acetylation of this ketone to the known 20,21-diacetate (**8**).[12] The stereochemistry here also is 11β,17α,20α.

Mechanism.—While we do not have any rate data and therefore do not know the reagents entering the rate-determining step, there seems to be no reason to postulate a mechanism different from that postulated by Westheimer[13] for alcohol oxidations.



cis ditertiary vicinal glycols are known to be cleaved quantitatively by chromic acid and a cyclic mechanism



is postulated for this process.[13] While this mechanism is reasonable for *cis* ditertiary glycols, it would be difficult to rationalize the effect of manganous ions in preventing cleavage in the secondary–tertiary glycols reported in this communication.

The product distribution in the oxidation of open-chain (pinacol)[7] and cyclic ditertiary glycols[6] has been studied. However, little appears to be known concerning the effects of manganese(II) on the product distribution in the oxidation of other vicinal glycols.

Acetate Location.—Gardi and co-workers[10,14] have reported that acid hydrolysis of 17α,20α,21-trihydroxypregna-1,4-diene-3,11-dione 17α,21-cyclopentanonide 20-acetate, in addition to removing the ketal, leads to migration of the 20-acetate to the 21 position. In addition to this rearrangement, Lewbart and Schneider[15] noted that a 20β-acetoxy-21-ol in the pregnane series yielded, on oxidation, the 20-keto 21-acetate. In view of these rearrangements, and in order to prove by nonchemical means the position of the acetate group in this series of compounds, an nmr study was made.

The basis for this study is the known shift of the position of absorption of hydrogen(s) α to an alcohol on acetylation.[16] In general, acetylation of a primary alcohol causes a downfield shift of about 30 cps, while acetylation of a secondary alcohol shifts the α-hydrogen absorption by 60–70 cps downfield.

We used 17α,20α,21-trihydroxy-4-pregnene-3,11-dione (**13**) as a reference compound and determined the shift of the C-20 and C-21 hydrogens caused by monoacetylation at C-20 or C-21 and diacetylation at C-20 and C-21. Table II shows the results. Monoacetylation causes both the 20 and 21 hydrogen absorptions to move downfield, one of them moves by 26 and the other by 44 cps. On diacetylation the absorptions which were shifted 26 are shifted downfield a further 15 cps and those shifted 44 are shifted a further 62 cps. This last shift shows that the second acetate went in at the 20 position and therefore the first acetate must be at the 21 position. These assignments are in agreement with those made by Gardi.[10]

(8) J. A. Hogg, *et al.*, *J. Am. Chem. Soc.*, **77**, 4438 (1955).
(9) B. H. Walker and I. B. Forsblad, U. S. Patent 3,057,884 (1962).
(10) R. Gardi, R. Vitali, A. Ercoli, and W. Klyne, *Tetrahedron*, **21**, 179 (1965).
(11) R. Gardi, R. Vitali, A. Ercoli, and W. Klyne, *Tetrahedron Letters*, No. 5, 189 (1962).
(12) F. Carvajal, *et al. J. Org. Chem.*, **24**, 695 (1959).
(13) Reference 1a, pp 37, 53; ref 1b, p 162.
(14) R. Gardi, R. Vitali, and A. Ercoli, *Gazz. Chim. Ital.*, **93**, 1642 (1963).
(15) M. L. Lewbart and J. J. Schneider, *J. Org.Chem.*, **29**, 2559 (1964).
(16) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press Inc., New York, N. Y., 1959, p 55.

Experimental Section

All melting points are corrected; ultraviolet absorption spectra were observed (in ethanol) with a Cary recording spectrophotometer, Model 11; rotations were observed with a Rudolph polarimeter at concentrations of *ca.* 10 mg/ml; nmr spectra were recorded with a Varian A-60 recording spectrometer using tetramethyl silane (TMS) as an internal standard; infrared spectra were taken as Nujol mulls on a Perkin-Elmer Model 421 spectrophotometer. The complex multiplets resulting from the coupling of the 20 and 21 hydrogens are not included in the Experimental Section; thin layer chromatographs (tlc) were conducted on silica gel GF 254 (E. Merck A. G.). The yields quoted in this section are based on the weight of isolated product obtained by chromatography and/or crystallization. Where the mixtures were obtained as with transitional fractions on chromatography, these have been ignored unless a pure product could be isolated by crystallization. Our product composition is therefore not exact, but the probable error can be calculated from the data.

11β,17α,20α,21-Tetrahydroxy-4-pregnen-3-one 21-Acetate (Triol Acetate,[17] 1).—This material was prepared as recorded earlier.[8] It was purified by recrystallization from tetrahydrofuran and tetrahydrofuran–methanol: mp 225–227°; λ_{max} 243 mμ (ε 15,550); $[\alpha]^{23}D$ +92° ($CHCl_3$); nmr 65 (C-18–CH_3), 88 (C-19–CH_3), 123 (CH_3 of acetate), 258 (11α-H), 336 cps (4 H) [lit.[8] mp 228–229°, $[\alpha]^{23}D$ +89° (acetone)].

11β,17α,20α,21-Tetrahydroxy-4-pregnen-3-one 20,21-Diacetate (3).—A mixture of 3.0 g of **1**, 10 ml of pyridine and 10 ml of acetic anhydride was allowed to stand overnight, then added to 600 ml of ice–water and filtered, and the product was recrystallized from acetone–water and from benzene to give **3** (2.02 g): mp 203–205°; $[\alpha]^{23}D$ +44° (dioxane); λ_{max} 243 mμ (ε 15,800); nmr 68 (C-18–CH_3), 87 (C-19–CH_3), 121 and 125 (C-20 and C-21–CH_3 of acetate) 265 (11α-H), 341 cps (4 H) [lit.[11] mp 203–205°, $[\alpha]D$ +39° (dioxane)].

17α,20α,21-Trihydroxy-4-pregnene-3,11-dione 21-Acetate (5). Method A.—This compound was prepared in two different ways from **1**. To 107 g (0.264 mole) of **1** was added 1200 ml of acetone, 120 ml of water, 83 ml of pyridine, 1 ml of glacial acetic acid, and 93.6 g (0.526 mole) of N-bromosuccinimide. The solution was allowed to stand for 60 hr and then was diluted with 10 l. of water. The precipitate was filtered, washed, and dried to give 75 g of crude product. Recrystallization from methanol yielded 62 g (58% of theory) of **5**: mp 243–246°; $[\alpha]D$ +132° (dioxane); λ_{max} 238 mμ (ε 15,800); λ_{max} 3510 and 3320 (OH), 1730 (acetate), 1695 (ketone), 1655 and 1618 cm^{-1} (3-keto-Δ^4).

Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.11; H, 8.06.

Method B.—A solution of 6 g of manganous chloride tetrahydrate in 90 ml of dimethylformamide was prepared. To this was added 40 ml of a solution of chromic acid (26.72 g of chromium trioxide, 23 ml of concentrated sulfuric acid and 76 ml of water). On adding the chromic acid solution the temperature rose to 54° and was easily cooled with an ice bath to 21°. Compound **1** (10.00 g) was added portionwise over a 3-min period during which time the temperature rose to 32° and then subsided to 30° during the 10-min reaction period. Water (400 ml) was added over 6.5 min. The pearlescent slurry was cooled to 10°, filtered, washed well with water, and dried to give 7.62 g of **5**. Paper chromatography showed the material to be 92 to 96% pure. An analytical sample was prepared: mp 249–251.5°; $[\alpha]^{23}D$ +133° (dioxane); λ_{max} 238 mμ (ε 15,540); nmr 48 (C-18–CH_3), 86 (C-19–CH_3), 123 (CH_3 of C-21-acetate), 340 cps (4 H).

17α,20α,21-Trihydroxy-4-pregnene-3,11-dione 20,21-Diacetate (7).—A slurry was prepared of 20 ml of acetic anhydride, 25 ml of pyridine, and 5 g of the above 11-keto-21-acetate (**5**). After 30 min at room temperature the product crystallized from the nearly clear solution. After a further 3.5 hr it was added to 250 ml of ice and water, filtered, washed with water, and dried to give 5.69 g of crude **7**, mp 271–272.5°. Recrystallization from acetone yielded 4.76 g: mp 277–278.5°; λ_{max} 238 mμ (ε 15,900); λ_{max} 3440 (OH), 1725 and 1717 (acetate), 1700 (11-ketone), 1677 and 1620 (3-keto-Δ^4), 1252 and 1235 cm^{-1} (COC of acetate); nmr 52 (C-18–CH_3), 85 (C-19–CH_3), 122 and 127 (C-20- and C-21–CH_3 of acetate), 344 cps (4 H).

(17) Triol acetate (**1**) is commercially available from the Chemical Sales Department of The Upjohn Co.

17α,20α,21-Trihydroxy-4-pregnene-3,11-dione (13).—The 11-keto 21-acetate (**5**) (1.4 g) was hydrolyzed by suspending it in 125 ml of methanol, 10 ml of water, and about 2 g of potassium carbonate. After stirring for 4.5 hr under nitrogen, tlc (20% ethyl acetate benzene) showed the reaction to be complete. The solvents were removed *in vacuo* at room temperature and the product was filtered and washed with water. On recrystallization from methanol, **13**, which was one spot by thin layer chromatography, had mp 240–244°; λ_{max} 238 mμ (ε 15,600); $[\alpha]D$ +144° (ethanol); λ_{max} 3490, 3400, and 3320 (OH), 1700 (11 ketone), 1650 and 1610 cm^{-1} (3-keto-Δ^4); nmr 43 (C-18–CH_3), 84 (C-19–CH_3), 339 cps (4 H) [lit.[10] mp 239–241°, $[\alpha]D$ +143° (dioxane), λ_{max} 239 mμ (ε 14,500), λ_{max} 3560, 3400, 1706, 1663, 1620 cm^{-1}; also, lit.[18] mp 240–243°, $[\alpha]D$ +141 ± 4°].

11β,17α,20α,21-Tetrahydroxy-1,4-pregnadien-3-one 21-Acetate (Δ¹-Triol Acetate, 2).—This material has been prepared by the oxidative hydroxylation procedure of Miescher[19] (along with prednisolone acetate) using hydrogen peroxide and osmium tetroxide on 11β,21-dihydroxy-1,4,17(20)-*cis*-pregnatrien-3-one 21-acetate.[20] A second, and perhaps preferable route, to the compound is disclosed by Gardi.[10] Our best sample of **2** [mp 236–243° dec; $[\alpha]D$ +32° (ethanol); λ_{max} 244 mμ (ε 14,900); nmr 61 (C-18–CH_3), 86 (C-19–CH_3), 122 (C-21–CH_3 of acetate), 263 (11α hydrogen), 357 (4 H), 370 (C-2–H, doublet J = 10 cps; this doublet is further split by the C-4–H, J = 2 cps), 443 cps (C-1–H, doublet J = 10 cps)] contained prednisolone acetate (*ca.* 5%)[21] as an impurity.

11β,17α,20α,21-Tetrahydroxy-1,4-pregnadien-3-one 20,21-Diacetate (4).—The above monoacetate (**2**, 3.0 g), which was mixed with 10 ml of acetic anhydride and 10 ml of pyridine, stood at ambient temperature for 18 hr. Addition to water yielded a semicrystalline solid which was filtered and washed with water. Thin layer chromatography showed this to be a single component (silica gel, 100% ethyl acetate). Recrystallization from benzene gave a benzene solvate, (1.13 g), mp 109–111° dec, resolidifying and remelting at 197–198.5°. An infrared absorption at 680 cm^{-1} and a strong nmr signal at 442 cps confirmed this. Recrystallization from acetone–Skellysolve B)[22] (SSB) gave the diacetate: mp 224–226°; $[\alpha]^{23}D$ −6.4° (dioxane); nmr 70 (C-18–CH_3), 88 (C-19–CH_3), 122 and 126 (C-20– and C-21–CH_3 of an acetate), 268 (C-11α–H), 362 (C-4–H), 375 (C-2–H doublet, J = 10 cps, where the doublet is further split by the C-4–H, J = 2 cps), 436 cps (C-1–H, doublet J = 10 cps).

17α,20α,21-Trihydroxy-1,4-pregnadiene-3,11-dione 21-Acetate (6).—A mixture (107 g) of **2** and prednisolone acetate was oxidized by the procedure described for the preparation of **5** except that N-bromoacetamide was substituted for N-bromosuccinamide. The crude oil obtained on addition of water was extracted into ethyl acetate. The ethyl acetate solution was washed with salt solutions, dried over sodium sulfate, and concentrated to give 35 g of crude solids from which after several recrystallizations from acetone a pure sample of **6** was obtained: mp 234–237°; $[\alpha]D$ +120° ($CHCl_3$); λ_{max} 239 mμ (ε 15,450); nmr 50 (C-18–CH_3), 87 (C-19–CH_3), 123 (C-21–CH_3 of acetate), 362 (C-4–H and one-half of the doublet of the C-2–H), 367 (C-2–H, doublet J = 10 cps further split by the C-4–H, J = 2 cps[23]), 461 cps (C-1–H, doublet J = 10 cps) [lit.[11] mp 233–235° $[\alpha]D$ +103° (dioxane)].

17α,20α,21-Trihydroxy-1,4-pregnadiene-3,11-dione 20,21-Diacetate (8).—A solution of 1.0 g of **6**, 10 ml of pyridine, and 5 ml of acetic anhydride was maintained at room temperature for 18 hr, added to water, filtered, washed, and dried. Recrystallization of the 245–252° melting crude 6 from acetone gave 640 mg: mp 252–255°; $[\alpha]$ +76.5° (dioxane); λ_{max} 238 mμ (ε 15,090); nmr 53 (C-18–CH_3), 87 (C-19–CH_3), 121 and 127 (C-20– and C-21–CH_3 of acetate), 366 (C-4–H and one-half of C-2–H doublet), 372 (C-2–H doublet, J = 10 cps further split by the C-4–H, J = 2 cps), 463 cps (C-1–H, doublet J = 10

(18) R. Naher and A. Wettstein, *Helv. Chim. Acta*, **39**, 2062 (1956).

(19) Miescher and J. Schmidlin, *ibid.*, **33**, 1840 (1950).

(20) J. A. Hogg, *et al.*, *J. Am. Chem. Soc.*, **77**, 4438 (1955); see also, J. A. Hogg, B. J. Magerlein, and J. Korman, *Steroids*, **3**, 189 (1964).

(21) Assayed by tetrazolium (red) assay: W. J. Mador and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).

(22) A saturated hydrocarbon fraction, bp 60–71°, available from the Skelly Oil Co., Kansas City, Mo.

(23) For another example of this C_2–C_4 coupling through the 3 ketone, see N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 124.

cps) [lit.[12] mp 250–251° dec and 267–270° dec, $[\alpha]^{25}D$ +75° (dioxane)].

Oxidation of 1 in the Presence of Manganous Nitrate.—To 10.000 g of **1** was added 90 ml of glacial acetic acid, 10 ml of water, and 20 ml of manganous nitrate solution (500 g of manganous nitrate/l.). The nearly homogeneous reaction mixture was cooled to 10° and a solution of 10.688 g of chromium trioxide dissolved in 30.4 ml of water was added during 3 min. The temperature of the reaction mixture rose to and was held at 30° during the oxidant addition. When the oxidant was all added the reaction mixture was cooled to 10° (ice bath) and 6.0 ml of concentrated sulfuric acid was added during 1 min. The exothermic reaction was allowed to warm to 30° and maintained there for 8 min after which 400 ml of water was added over the next 10 min. The resultant slurry was cooled to 10° and maintained there for 10 min, filtered, and washed with 600 ml of water to give a white cake. The precipitate was dried in a vacuum oven at 60° to give 5.433 g, mp 228–231° (crude **9**).

The filtrate was extracted with five 100-ml portions of an equal volume mixture of methylene chloride and ethyl acetate. The combined extracts were washed with 250 ml of water and taken to dryness to give 3.865 g (filtrate extract).

Crude **9** (5.350 g) was dissolved in 200 ml of acetone, filtered, concentrated to 85 ml, cooled for 18 hr at 5°, filtered, and dried at 50° (20 mm) to give 4.893 g of an acetone solvate of cortisone acetate (**9**): mp 237.5–239°; $[\alpha]D$ +196° (dioxane); on drying at 80° (0.01 mm) the $[\alpha]D$ was +212° (dioxane). By papergram assay this material was estimated to be >98% pure. [A previous sample of **9** prepared in this way, mp 240–243°, $[\alpha]D$ +213° (dioxane), λ_{max} 238 mμ (ε 15,200), had an identical infrared spectrum with the above sample.] The filtrate extract and the acetone solution from recrystallization of the crude **9** were combined, concentrated to dryness, dissolved in methylene chloride, and chromatographed on 250 g of Florisil.[24] The products were eluted in 500-ml fractions with increasing amounts of acetone in methylene chloride (see Table III).

TABLE III

Fractions	% acetone	Wt of product	Product
1–4	2–3	...	...
4–12	4–5	1.528 g	**11**
13	5	81 mg	Mixture
14–18	5–10	888 mg	**9**

Compound **11** (1.528 g) on recrystallization from acetone gave 1.086 g, mp 222–224° [an analytical sample prepared in an earlier experiment by this procedure melted at 221.5–224.5°; $[\alpha]^{23}D$ +304° ($CHCl_3$), $[\alpha]^{23}D$ +287 (ethanol); λ_{max} 238 (ε 15,322)]. Recrystallization of **9** (888 mg) from acetone gave 516 mg of **9**, mp 243–246°. The yield of **9** from **1** in the presence of manganous nitrate is 50% (assuming 1 mole of acetone/mole of steroid) and the yield of **11** is 21%.

Oxidation of 1 in the Absence of Manganous Salts.—In exactly the same manner as in the previous experiment, a 10.000-g sample of **1** was oxidized but without added manganese. In this case, the precipitated solids weighed 6.1 g and melted at 197–214°. Thin layer chromatography showed this material to be mostly **11**. Extraction of the mother liquor gave 6.0 g of moist solid on concentration.

The total precipitate and extract were combined and chromatographed on 250 g of Florisil.[24] The steroid dissolved in methylene chloride was placed on the column and eluted in 500-ml fractions with methylene chloride containing increasing amounts of acetone (see Table IV).

TABLE IV

Fractions	Solvent	Wt	Product
1–3	1–2% acetone	7.5 mg	...
4–14	2–4% acetone	5.294 g	Nearly pure **11**
15–16	6% acetone	0.544 mg	Mixture **11** and **9**
17–21	8% acetone	1.055 g	**9**

Compound **11** (5.29 g) was recrystallized from acetone to give 4.257 g, mp 223–226°. The mixture from fractions 15 and 16 gave on recrystallization from acetone 326 mg of **9**, mp 245–249°. The compound **9** fraction on recrystallization from acetone gave 656 mg, mp 246–249°. Without manganese, cortisone acetate (**9**) is obtained in 14% yield and adrenosterone (**11**) is obtained in 72% yield.

Oxidation of 2 in the Presence of Manganous Nitrate.—To 67.5 ml of glacial acetic acid was added 10.000 g of **2**, 10 ml of water, and 10 ml of 50% manganous nitrate solution. The mixture was cooled to 10° and a solution of chromium trioxide (8.684 g) in water (30.4 ml) was added over about 30 sec. The temperature rose over 4.5 min to 28°. Cooling to 10° with an ice bath, (3 min), was followed by the addition (1 min) of 9.5 ml of concentrated sulfuric acid. The temperature rose to 31° during the acid addition and was cooled to, and maintained at, 25° for 8 min. The product was precipitated by the addition of 400 ml of water (10 min) cooled to and maintained at 10° for 14 min. The product was filtered, washed with water, (*about* 600 ml) and dried *in vacuo* at 60°. The yield was 5.870 g of crude **10**. This material was estimated to be about 95% pure by tlc with a trace of a more polar impurity. Crude **10** (5.85 g) was recrystallized from acetone (dissolved in 125 ml, recovered from 35 ml) to give 4.608 g of **10**: mp 233–235°; $[\alpha]D$ +185° (dioxane); λ_{max} 240 mμ (ε 14,600); and an infrared spectrum identical with that of an authentic sample.

The filtrate which yielded crude **10** was extracted in the same way as described for the oxidation of **1**. This extract was combined with the acetone filtrate from which the **10** had been crystallized. This combined solution was concentrated to dryness, dissolved in methylene chloride, and chromatographed on 250 g of Florisil eluting in 500-ml fractions with methylene chloride containing increasing amounts of acetone (see Table V).

TABLE V

Fractions	% acetone	Wt	Products
1–6	0–3	...	...
7–16	3	1.648 g	**12**
17–21	3–4	269 mg	Mixture of **10** and **12**
22–28	6–10	980 mg	**10**

Compound **12** (1.648 g) was recrystallized from acetone to give 1.140 g, mp 197–199.5°. Compound **10** (980 mg) was recrystallized from acetone to give 699 mg, mp 234–237°. Thus, from 10 g of **1** we get a 56% yield of prednisone acetate (**10**) and a 22% yield of Δ^1-adrenosterone (**12**) in the presence of added manganese.

Oxidation of 2 in the Absence of Manganese Salts.—Using the same procedure and amounts as shown above, but without added manganese, 6.012 g of solid was obtained. This was estimated by tlc to be about 60:40 **10** to **12**. The filtrate was extracted as shown under the oxidation of **1** and the whole mixture was chromatographed.

The chromatography was conducted on 500 g of Florisil. The steroid mixture was dissolved in 100 ml of methylene chloride and eluted with methylene chloride containing increasing proportions of acetone (500-ml fractions; see Table VI).

TABLE VI

Fractions	Solvent	Wt	Product
1–7	1–3% acetone	64 mg	Oil
8–23	3 and 4% acetone	4.243 g	**12**
24–29	4 and 5% acetone	484 mg	Mixture of **10** and **12**
30–48	5–15% acetone	2.772 g	**10**

Compound **12** (4.243 g) was recrystallized from acetone to give 3.07 g of material (one spot tlc), mp 197–198.5°. Compound **10** (2.772 g) was recrystallized from acetone to give material (1.684 g), mp 235–237.5° (one spot by tlc). Thus, in the absence of manganese, a 57% yield of Δ^1-adrenosterone (**12**) and a 28% yield of prednisone acetate (**10**) was obtained.

Oxidation of 5 in the Presence of Manganese Nitrate.—A slurry was prepared of 1.000 g of **5**, 9.0 ml of glacial acetic acid, 1.0 ml of water, and 2.0 ml of manganous nitrate (50% solution). To the stirred slurry was added over 1.25 min, a solution of 0.91 g of chromium trioxide in 3.0 ml of water. The temperature rose from 24 to 25°. The reaction was allowed to continue for 2.75 min then cooled to +10° (1.25 min). Sulfuric acid (0.5 ml)

(24) A synthetic magnesia–silica gel manufactured by the Floridin Co., Warren, Pa.

was added over 0.5 min and the reaction was allowed to proceed for 8 min during which time the temperature rose from 10 to 24°. Next, 40 ml of water was added over 2.5 min. The slurry was cooled to 9° over the next 7 min. In order to get as quantitative a recovery as possible, the 9 was not filtered, but the whole reaction mixture was extracted with four 50-ml portions of methylene chloride which was washed with 50 ml of saturated sodium bicarbonate and half-saturated sodium chloride solutions. After removal of the solvent, under reduced pressure, the residue was dried under 0.01 mm at 60° to give 791 mg of solids. Analysis by quantitative tlc[25] showed this material to be composed of 13% 11 and 60% 9 by weight based on 791 mg of material. Based on 1.000 g of 5 this corresponds to a 48% yield of cortisone acetate (9) and a 14% yield of adrenosterone (11).

Oxidation of 5 in the Absence of Manganese.—Using exactly the same procedures and amounts as in the experiment reported above but without added manganous nitrate gave 764 mg of product. Quantitative tlc showed that 54% of the weight of this product was adrenosterone and 39% cortisone. These values correspond to a yield of 56% of adrenosterone (11) and 30% of cortisone acetate (9).

(25) Private communication, E. J. Kubiak and A. J. Taraska, Control Research and Development Department, The Upjohn Co.

Oxidation of 1 in the Presence of Cerous Nitrate.—A slurry of 10.00 g of 1, 67.5 ml glacial acetic acid, 24.26 g cerous nitrate hexahydrate, and 30.1 ml of water was prepared. To the slurry was added over 4 min an aqueous solution of 12.02 g of chromium trioxide and 6.9 ml of sulfuric acid (20.4 ml of water). An immediate precipitate formed on mixing the oxidant with the slurry. The temperature rose to 30° and was maintained there by cooling during the 8-min reaction. Over the next 10 min, 300 ml of water was added. The slurry was cooled to 10°, filtered, washed white with water (600 ml), and dried to give 4.28 g of pure cortisone acetate (9) as determined by paper chromatography.

Registry No.—**1**, 10026-44-5; **2**, 2871-71-8; **5**, 7738-89-8; **6**, 1758-06-1; **3**, 2481-66-5; **4**, 2483-33-2; **7**, 2300-23-4; **8**, 2483-34-3; **9**, 50-04-4; **10**, 125-10-0; **11**, 382-45-6; **12**, 7738-93-4; **13**, 566-38-1; chromic acid, 7738-94-5.

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On the Reactions of Carbonyl Compounds with N-Salicylideneglycinatoaquocopper(II). Syntheses of β-Hydroxy α-Amino Acid from Glycine[1]

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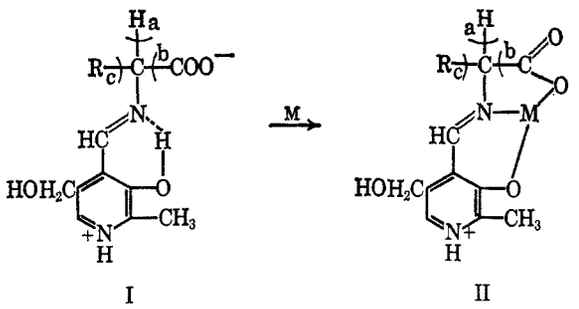
Several forms of N-salicylideneglycinatoaquocopper(II) were synthesized. These complexes were found to be the same in structure except for combining with water. Carbonyl compounds react rather easily with these complexes to form N-salicylidene-β-hydroxy amino acid–copper complex under conditions of aqueous solution, at room temperature (25 ± 1°) and at neutral to weakly alkaline pH. Threonine, β-phenylserine, β-hydroxyaspartic acid, and serine were synthesized. The yields dependent on reaction time and the ratios of *threo* and *erythro* isomers were studied. The reactions are similar to those nonenzymatically catalyzed by pyridoxal and the complexes could be regarded as the benzene analogs of pyridoxalamino acid complexes.

It has been found that many naturally occurring enzymes contain pyridoxal 5-phosphate as a coenzyme.[2] Nonenzymatic reactions catalyzed by pyridoxal or pyridoxamine have been studied extensively by Metzler and Snell.[3] The chemistry of pyridoxal was reviewed by Snell,[3] Westheimer,[4] and Braunstein.[5] Aldimines (I) are formed from pyridoxal with amino acids, and their metal chelating compounds will be illustrated as II in Scheme I.[3c]

In both structures I and II, chemical bonds a, b, and c combined with the α-carbon atom of the amino acid could be weakened by the aldimine formation. In structure I, bond b might be the weakest and decarboxylation might take place. On the other hand, in structure II, the carboxyl group could be stabilized by the formation of a chelate, and bond c could be

SCHEME I



weakened. Metzler, Longenecker, Ikawa, and Snell[6,7] reported the reversible catalytic cleavage of β-hydroxy α-amino acids using pyridoxal and metal ions by heating in aqueous solution. These nonenzymatic reactions catalyzed by pyridoxal seem to be similar in reaction mechanism to those catalyzed by enzymes which contain pyridoxal as a coenzyme. Ikawa and Snell[8] have studied the chemical properties of benzene analogs of pyridoxal with amino acids. They found 4-nitrosalicyaldehyde simulates pyridoxal in its reactions with

(1) Contribution No. 078 of the Institute of Molecular Evolution, University of Miami.

(2) Some of the pyridoxal-containing enzymes are amino acid decarboxylase, transaminase, tryptophanase, kynureninase, tyrosinase, serine deaminase, cysteine desulfhydrase, and hydroxy amino acid aldolase.

(3) (a) E. E. Snell, Special Lectures in Biochemistry," University College, London, H. K. Lewis, Distributors, 1954–1955, pp 1–16; (b) E. E. Snell, *Vitamins Hormones*, **16**, 78 (1960); (c) "Chemical and Biological Aspects of Pyridoxal Catalysis," E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fanelli, Ed., The Macmillan Co., New York, N. Y., 1963, p 1.

(4) F. H. Westheimer, "The Enzymes," Vol. 1, P. D. Boyer, H. Lardy, and K. Myrback, Ed., Academic Press Inc., New York, N. Y., 1959, p 259.

(5) A. E. Braunstein, "The Enzymes," Vol. 2, Academic Press Inc., New York, N. Y., 1960, p 113.

(6) D. E. Metzler, J. B. Longenecker, and E. E. Snell, *J. Am. Chem. Soc.*, **75**, 2786 (1953); **76**, 639 (1954).

(7) D. E. Metzler, M. Ikawa, and E. E. Snell, *ibid.*, **76**, 648 (1954).

(8) M. Ikawa and E. E. Snell, *ibid.*, **76**, 653 (1954).