ther HCl was evolved, was found to contain somewhat above 12% Cl.

Reyerson and Bemmels[2] show that the adsorption of hydrogen chloride on silica gel is normal in magnitude, reversible and decreases with elevation of temperature. It follows that Rao and Rao are in error in considering the Cl retained after CCl_4 treatment to be present as HCl. Rather, the product must be regarded as of the nature of an oxychloride of silicon with chlorine chemically bound to silicon, since HCl as such would not remain in the gel under the conditions of the preparation.

[2] L. H. Reyerson and C. Bemmels, *J. Phys. Chem.*, **46**, 35 (1942).

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Reversible Bleaching of Chlorophyll by Metallic Salts

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RECEIVED JANUARY 3, 1953

The interaction of chlorophyll with metallic salts possessing oxidizing and reducing properties is of importance in considering the participation of chlorophyll in oxidation–reduction systems. Addition of an oxidizing salt to an alcoholic or acetone solution of chlorophyll a or b causes bleaching to a straw yellow color within one or two seconds. Immediate subsequent addition of a reducing salt causes a reversion to green. The reversible reaction

$$\text{Chlorophyll} \underset{\text{reducing salt}}{\overset{\text{oxidizing salt}}{\rightleftarrows}} \text{``oxychlorophyll''} \quad (1)$$

has been proposed, where "oxychlorophyll" is merely the designation of the unstable bleached form of chlorophyll.[1] The reversibility of (1) has not received complete credence among biochemists as the absorption spectra of the regenerated green solutions were significantly different from the initial spectra in some cases where regeneration of chlorophyll was claimed.

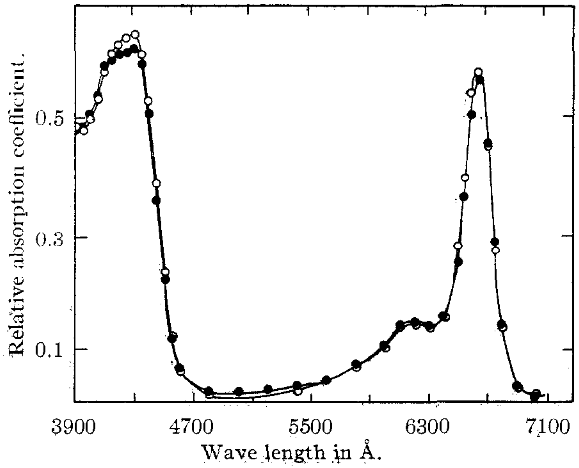

Fig. 1.—Regeneration of the spectrum of non-allomerized chlorophyll a: —●—, 5×10^{-6} m. chlorophyll a in methanol; —○—, after bleaching with ferric chloride and regeneration by cuprous chloride.

The data now presented confirm that chlorophyll undergoes a reversible reaction of type (1) within the accuracy of modern spectrophotometric methods, after allowance for a small amount of allomerized chlorophyll also produced. The reversion to green on addition of sodium chloride or standing exposed to air is not regeneration of chlorophyll but formation of allomerized chlorophyll. The reversion of color by hydroquinone is by production of yet another green compound.

Experimental Methods and Materials.—Chlorophylls a and b were prepared by a modification of Zscheile and Comar's method.[2] Purified chlorophyll was kept in ether at −10° in the dark till required, and then transferred to methanol by evaporating and condensing on a vacuum apparatus with avoidance of complete drying-off of solvent. Spectral measurements were made using conventional 1-cm. cells in a Beckman spectrophotometer. Methanolic solutions of salts were added directly to chlorophyll solutions in the Beckman holder from syringes.

Experimental Results and Discussion.—The spectrum after bleaching by ferric chloride and regeneration by cuprous chloride is illustrated in Fig. 1. Apart from the small increase in the main violet maximum and reduction in the minor violet maximum, typical of allomerization,[3] the chlorophyll was almost quantitatively regenerated. A chlorophyll solution left at room temperature till partially allomerized also underwent regeneration of spectrum except for slight further allomerization. The sensitivity of the bleached compound to traces of water was confirmed[1]; water of hydration of 10^{-5} mole/l. ferric chloride caused a marked loss of chlorophyll a on regeneration.

The bleached solution returned to green on standing. However, the spectrum was characteristic of allomerized chlorophyll.[3] (Figure 5 of the original paper[1] shows that the spectra of allomerized chlorophyll was also observed, though its spectrum at that time had not been independently characterized.) This removes the main, and recognized,[1] anomaly of reversion on standing.

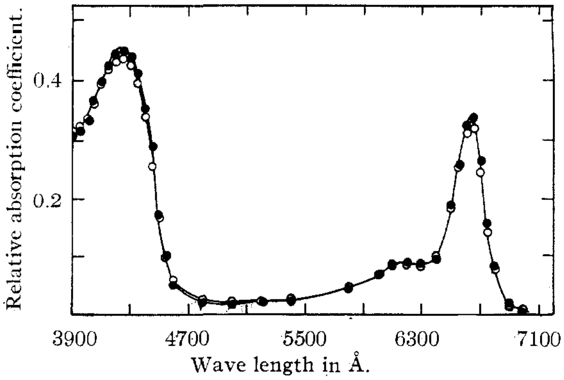

Fig. 2.—Regeneration of the spectrum of partially-allomerized chlorophyll a: —○—, partially-allomerized chlorophyll a in methanol; —●—, after bleaching with ferric chloride and regeneration by cuprous chloride.

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[1] E. Rabinowitch and J. Weiss, *Proc. Roy. Soc. (London)*, **A162**, 2511 (1937).

[2] R. Livingston, D. Sickel and A. Uchiyama, *J. Phys. Colloid Chem.*, **51**, 777 (1947).

[3] E. I. Rabinowitch, "Photosynthesis," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 613.

There is as yet no complete confirmation that the bleached compound is an oxidation product of chlorophyll. An alternative explanation is that complex formation with Fe^{+++} occurs.[4] The interpretation of the bleaching as formation of an intermediate oxidation product is supported by a similar transitory bleaching by bromine and iodine and the production of allomerized chlorophyll on allowing the ferric salt to react without reversion. The effect of non-oxidizing salts[4] is probably similar to the acceleration of allomerization by lanthanum chloride[3]; dissolved oxygen was necessary and again an initial partial bleaching was observed.

This investigation was sponsored by the O.N.R. (Contract NR-ori-212 for the year 1948) at the University of Minnesota.

[4] M. S. Ashkinazi, T. S. Glikman and B. J. Dain, *Compt. rend. Acad. Sci., U. R. S. S.*, **73**, 743 (1950).

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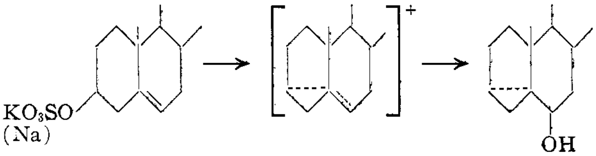
The Origin of 3,5-Cycloandrostan-6β-ol-17-one (i-Androsten-6β-ol-17-one) in Urinary Extracts

BY SYLVIA TEICH, JEAN ROGERS, SEYMOUR LIEBERMAN, LEWIS L. ENGEL AND JOHN W. DAVIS

RECEIVED DECEMBER 8, 1952

In 1948 Dingemanse, Huis in't Veld and Hartogh-Katz[1] reported the isolation from human urine of a new 17-ketosteroid, $C_{19}H_{28}O_2$, m.p. 140.5–141°, which shortly thereafter was identified[2–4] as 3,5-cycloandrostan-6β-ol-17-one (III).[5] Because III was readily converted even at room temperature by aqueous hydrochloric acid into 3-chloro-Δ^5-androsten-17-one (IV) and dehydroisoandrosterone (V), it was concluded[2] that the i-steroid was in fact the precursor of these familiar urinary products. III was isolated from urine only when neutral urine heated on a steam-bath was extracted continuously with benzene, a procedure which was postulated[6] to cause the hydrolysis of a "certain conjugate" of III without effecting its subsequent conversion to IV or V. The failure of previous workers to isolate any i-steroid was ascribed to the use of methods which involved acid hydrolysis either at room or elevated temperatures.[2]



I II III

This paper reports the conversion of sodium and potassium dehydroisoandrosterone sulfate (I) to 3,5-cycloandrostan-6β-ol-17-one (III) using the conditions employed by Dingemanse, *et al.*,[1,6] for the isolation of III from urine, *i.e.*, heating a neutral aqueous solution covered with a layer of benzene and also by refluxing solutions buffered at various *p*H values under toluene. Since I has been isolated from urine[7] and is the most important known urinary conjugate of dehydroisoandrosterone, its conversion to III can satisfactorily account for all the observed results without postulating that the i-steroid is a naturally occurring urinary metabolite which serves as a precursor of dehydroisoandrosterone. The equivocal manner by which III was isolated from urine leaves the existence of the i-steroid as a genuine steroid metabolite in doubt.[8]

The potassium dehydroisoandrosterone sulfate employed in our experiments was prepared[9] by the the action of pyridine–sulfur trioxide[10] on dehydroisoandrosterone. To ensure that the product of this reaction was indeed the 3-sulfate of the normal steroid and not the 6-sulfate of the i-compound resort has been taken to the Method of Molecular Rotation Differences.[11] Earlier data indicate that a sulfate group makes only a small negative contribution to the M_D value. Thus, the ΔM_D(M–O–SO$_2$O–) calculated from sodium cholestan-3β-ol sulfate (M_D +81)[9] and cholestan-3β-ol (M_D +113) is −32, and that calculated from sodium cholestan-3α-ol sulfate (M_D +74)[9] and cholestan-3α-ol (M_D +132) is −58. It seems certain, therefore, that the sulfate group in I is at C_3 since the ΔM_D(M–O–SO$_2$O–) calculated from potassium dehydroisoandrosterone sulfate (M_D +28) and dehydroisoandrosterone (M_D +29) is −1. On the other hand, the M_D of the 6-sulfate of the i-steroid (III) would be expected to be a large positive value, only slightly less positive than that of the M_D of the corresponding alcohol (+351).

The hydrolysis of steroid sulfates has been studied under a variety of conditions and the results can be summarized as follows:

At *room temperature* the sulfates can be cleaved under acidic conditions and this has been shown[9] to involve the splitting of the S–O bond of the sulfate, affording the alcohol with retention of configuration.

When *elevated temperatures* are employed, the C–O bond of the sulfate ester linkage may dissociate to give a resonating hybrid (II), such as described by

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[1] E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *Nature*, **161**, 848 (1948).

[2] E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *ibid.*, **162**, 492 (1948).

[3] D. H. R. Barton and W. Klyne, *ibid.*, **162**, 494 (1948).

[4] E. Dingemanse and L. G. Huis in't Veld, *J. Biol. Chem.*, **195**, 827 (1952).

[5] It has been postulated on theoretical and molecular rotation considerations that the i-steroids formed by rearrangement of the 3-tosylates have a 6β-configuration; ((a) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948); C. W. Shoppee, *Bull. soc. chim.*, **18**, 120 (1951)). In a recent comprehensive review of the stereochemistry and mechanism of this rearrangement convincing chemical evidence (b) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 3361 (1952)) has been presented which further substantiates this assignment. Based on reactions whose stereochemical course has not been clearly elucidated, Wallis and co-workers ((c) A. F. Wagner, N. E. Wolff and E. S. Wallis, *J. Org. Chem.*, **17**, 529 (1952)) have assigned the opposite configuration to the i-steroid.

[6] E. Dingemanse, L. G. Huis in't Veld and S. Hartogh-Katz, *J. Clin. Endocrin. and Metab.*, **12**, 66 (1952).

[7] P. Munson, T. F. Gallagher and F. C. Koch, *J. Biol. Chem.*, **152**, 67 (1944).

[8] H. L. Mason and W. W. Engstrom (*Physiol. Rev.*, **30**, 321 (1950)) have already called attention to this and have suggested that the procedure used for extraction might be responsible for the conversion of I to III.

[9] S. Lieberman, L. B. Hariton and D. K. Fukushima, THIS JOURNAL, **70**, 1427 (1948).

[10] A. E. Sobel and P. E. Spoerri, *ibid.*, **63**, 1259 (1941).

[11] D. H. R. Barton and W. Klyne, *Chemistry and Industry*, 755 (1948).