[CONTRIBUTION FROM THE RESEARCH LABORATORIES, CHEMICAL DIVISION, MERCK & CO., INC., RAHWAY, N. J.]

Studies on the Chemistry of Aldosterone

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RECEIVED OCTOBER 18, 1954

Degradative studies on aldosterone (electrocortin) presented here are in accord with the structure 11β,21-dihydroxy-3,20-diketo-4-pregnene-18-al proposed by Simpson, *et al.*,[5] for the new mineral-regulating adrenal hormone. Some additional transformations and degradation products of aldosterone are described. These reactions concerned primarily the nature of the oxygen function at C_{18}, and included glycoside formation, acylation and hydrolytic reactions and borohydride reduction.

Reports from other laboratories concerning structural features of aldosterone (electrocortin)[1-4] and the recent complete elucidation of its structure as 11β,21-dihydroxy-3,20-diketo-4-pregnene-18-al[5] (Ia) have prompted us to record details of our study on the chemistry of this substance. The isolation of crystalline aldosterone in this Laboratory is described in a preceding paper.[6] The conclusions drawn from chemical studies reported here are in complete accord with the structure advanced by the British–Swiss investigators. Our structural studies on aldosterone paralleled in part those of Simpson and co-workers[5] but also included some new degradation reactions and products.

Microanalytical data suggested the empirical formula $C_{21}H_{28-30}O_5$ for the new hormone; ultraviolet and infrared spectra,[6] optical rotation,[6] reduction of tetrazolium salts[7] and the consumption of one molecular equivalent of periodate with liberation of formaldehyde all indicated that aldosterone might be a 21-hydroxy-3,20-diketo-4-pregnene with two additional oxygen atoms. The α-ketol side chain and the Δ^4-3-keto structure were also suggested by the earlier investigators.[1-4] The C_{20}-carbonyl group in adrenal steroids characteristically contributes an intense absorption maximum in the 5.85 μ region of the infrared spectrum.[8] The infrared spectra of some samples of crystalline aldosterone had very weak maxima at 5.87 μ; other samples had no maximum at that position. Completely acetylated aldosterone, however, had an infrared absorption maximum in the saturated carbonyl region (5.76 μ in carbon tetrachloride solution) as well as acetate (5.69 μ) and conjugated carbonyl (5.94 μ) maxima. It thus appeared that form Ib was important in aldosterone as well as form Ic mentioned by Simpson, *et al.*[5]

Periodic acid oxidation of aldosterone yielded formaldehyde and a stable crystalline product (II) which was inactive in the urinary sodium retention bioassay.[6] This substance had infrared absorption bands attributable to the conjugated carbonyl system and to a γ-lactone grouping but did not have hydroxyl group absorption. These products were obtained similarly by the European workers.[5] Lactone II was readily opened by alkali to yield crystalline lactol-acid III, from which crystalline methyl ester IIIa was obtained by treatment with diazomethane. Sublimation of the lactol-acid resulted in regeneration of the original lactone. Potentiometeric titration of III gave a value in agreement with that required by the postulated structure. Oxidation of lactol-acid III with chromic acid–pyridine complex[9] gave crystalline γ-lactone-acid IV. The nature of the functional groups in lactone-acid IV was deduced from the infrared absorption bands at 5.64, 5.75 and 6.11 μ in the spectrum of the free acid and at 5.69, 6.03 and 6.41 μ in the spectrum of the sodium salt of the acid. Again, the compound showed one carboxyl group by potentiometric titration. Oxygen analysis indicated the presence of five oxygen atoms. The presence of an oxygen function at C_{18} in aldosterone had been suggested by the lack of a well-defined C_{20}-carbonyl group absorption maximum in the infrared; the conversion of lactol-acid III to lactone-acid IV by chromic acid oxidation could be explained only by a C_{18}-oxygenated structure. It is of interest that Simpson and co-workers[5] degraded aldosterone to γ-lactone-acid IV by a different series of reactions.

The lactol (glycosidic) nature of the fourth oxygen atom of aldosterone, assigned to position 18, was confirmed by a number of additional isolated observations. No hydroxyl group infrared absorption could be detected in lactone II, which resulted from periodate oxidation of aldosterone, nor was the compound altered by attempted acetylation or mild chromic acid oxidation. The absence of any identifiable oxygen function in II other than conjugated carbonyl and γ-lactone suggested that the fifth oxygen atom of the parent compound, or the fourth and unidentified oxygen of lactone II, was attached to two carbon atoms either as an epoxide or as the ring oxygen atom of a cyclic hemiacetal structure. Lactol-acid III was also converted to lactone-acid IV, as indicated by paper chromatographic analysis of the reaction products, by bromine under conditions used for the preparation of aldonic acid lactones. Aldosterone diacetate V was converted to the 21-monoacetate by very mild acid hydrolysis. Attempted reaction of lactol-acid methyl ester IIIa with methanesulfonyl chloride in pyridine led either to recovery of unchanged starting material or, with drastic conditions, to decomposition. A product

(1) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw and T. Reichstein, *Experientia*, **9**, 333 (1953).

(2) V. R. Mattox, H. L. Mason, A. Albert and C. F. Code, THIS JOURNAL, **75**, 4869 (1953).

(3) R. E. Knauff, E. D. Nielson and W. J. Haines, *ibid.*, **75**, 4868 (1953).

(4) S. A. Simpson and J. F. Tait, *Mem. Soc. Endocrinol.*, **2**, 9 (1953).

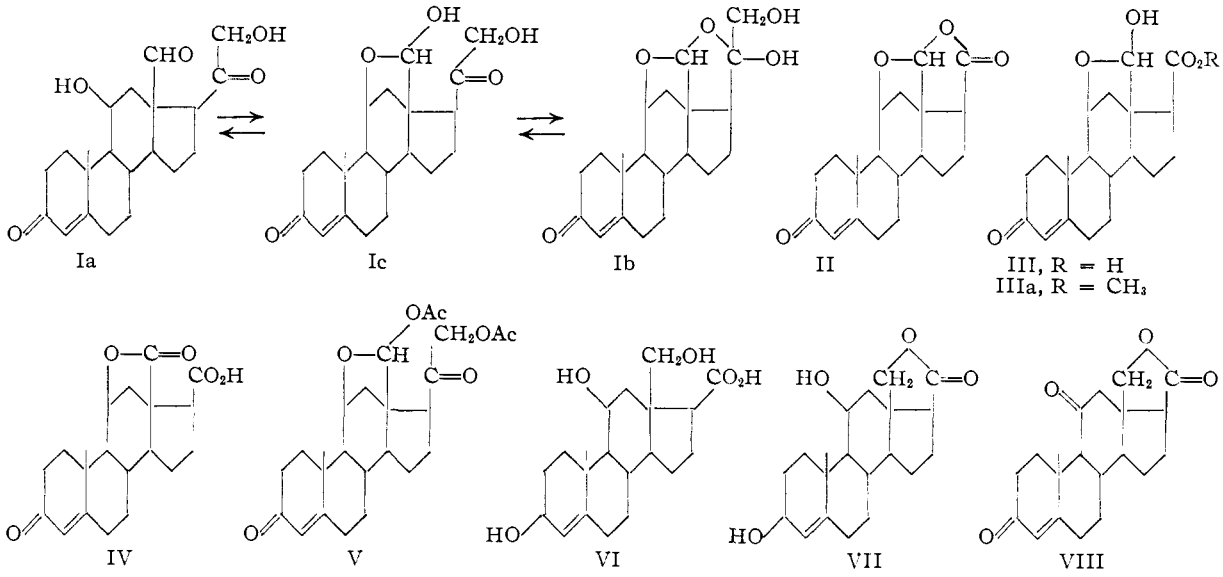
(5) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler, T. Reichstein, *Experientia*, **10**, 132 (1954); *Helv. Chim. Acta*, **37**, 1200 (1954).

(6) R. E. Harman, E. A. Ham, J. J. DeYoung, N. G. Brink and L. H. Sarett, THIS JOURNAL, **76**, 5035 (1954).

(7) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(8) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950).

(9) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).



which showed no hydroxyl group absorption in the infrared resulted when either lactone II or lactol-acid methyl ester IIIa was treated with anhydrous methanolic hydrogen chloride. Rationalization of these results required assignment of a lactol structure at C_{18}.

The point of attachment of the fifth oxygen atom of aldosterone appeared to be either C_8 or C_{11}, as judged from the γ-lactone character of lactone-acid IV. Attempts to open this lactone ring and so isolate the last oxygen as the hydroxyl group of an hydroxysuccinic acid were unsuccessful. Selection of C_{11} was made possible by a reaction sequence starting with sodium borohydride reduction of lactol-acid III. The crude reduction product, represented by structure VI, was heated to form dihydroxylactone VII. This neutral product was oxidized with chromium trioxide–pyridine complex to give crystalline diketolactone VIII. This diketolactone was characterized by its infrared absorption spectrum, which contained a strong saturated carbonyl group band at 5.89 μ in addition to absorption bands ascribable to conjugated carbonyl and γ-lactone groups.

The reactions of aldosterone described above led to the structure 11β,21-dihydroxy-3,20-diketo-4-pregnene-18-al for the hormone, based on the assumption of a 4-pregnene skeleton. The correctness of this assumption has of course been demonstrated by the English–Swiss workers,[5] who succeeded in degrading aldosterone to known steroid derivatives.

Mild acetylation of aldosterone gave high yields of the 21-acetate (m.p. 193–196.5°), which was as active as the free alcohol in the urinary sodium retention bioassay.[6] This compound appears to differ from the monoacetate of m.p. 217–219° obtained by Mattox, *et al.*,[2] by enzymatic hydrolysis of the diacetate.

Experimental

Melting points were determined on a micro hot-stage. Infrared data were obtained on a Baird Associates Infrared Recording Spectrophotometer Model B, or a Perkin–Elmer Infrared Spectrometer Model 12c when less than milligram quantities of sample were available.

The "chromatocoil" paper partition chromatography technique of Schwarz[10] was well suited to our purposes. The specially constructed chamber used by Schwarz was found unnecessary; satisfactory results were obtained when a 10-cm. desiccator (for up to 4 eighty-cm. strips) or a commercial baking dish (as many as 10 strips) with carefully ground-on cover, was used to hold the coil supports. A small beaker of each solvent phase, equipped with a T-shape wick, served to equilibrate the system. The dry strips (80 cm. × 1 cm. Whatman #1 paper) were wound on the coil support, spotted with test samples and allowed to equilibrate for $^1/_2$–1 hour. Mobile phase was then introduced to about 0.5-cm. depth and the system allowed to run for 16 hours at laboratory temperature. R_f values were reproducible under these operating conditions to about 0.1 unit.

Three solvent systems were required to handle effectively materials of the range of polarities encountered. In each system, 50% aqueous methanol was used as stationary phase. Mobile phases, and reference steroids for each system, were 10% benzene–hexane (11-desoxycorticosterone, R_f 0.60 ± 0.05), 40% benzene–hexane (11-dehydrocorticosterone, R_f 0.45 ± 0.05) and benzene (cortisone, R_f 0.45 ± 0.05). The mobility (R_{DOC}, R_A, R_E) of a substance is recorded as the ratio of the distance moved by the sample to the distance moved by the reference steroid, DOC, A or E. In general, 0.02–0.04 mg. of homogeneous material was sufficient for easy detection by ultraviolet scanner[11] and/or tetrazolium salt reduction.[7] When mixtures were analyzed by paper chromatography, an attempt was made to run sufficient material to provide at least 0.02 mg. of each compound present in significant amount.

Reactions were run in standard 15-ml. centrifuge cones. Work-up of reaction mixtures consisted of a series of 3 to 6 extractions with 1 ml. of ethyl acetate followed by back-washing with 1 ml. of water. A second set of extractions was generally used to ensure complete recovery of steroidal reaction products, and a third series also, if required.

Aldosterone.—The crystalline hormone was isolated as described in a previous publication,[6] in which are recorded also some of its physical properties. Analytical samples were dried in a weighing-pig at 100° in high vacuum, a procedure shown by bioassay, ultraviolet and infrared spectra, melting point and sulfuric acid chromogen to leave the compound unaltered.

Anal. Calcd. for $C_{21}H_{28}O_6$: C, 69.97; H, 7.83; O, 22.20. Found: C, 69.64, 69.45; H, 8.06, 7.86; O, 23.5.

Lactone II. (A) From Crystalline Aldosterone.—A solution of 25 mg. of periodic acid in 1 ml. of water was added to 8 mg. of aldosterone in 1.2 ml. of dioxane and the mixture kept at room temperature for two hours. Water (3 ml.) was then added and ethyl acetate extraction carried out.

(10) V. Schwarz, *Chemistry and Industry*, 102 (1953).

(11) W. J. Haines and N. A. Drake, *Federation Proc.*, **9**, 180 (1950).

There was obtained 7.3 mg. (quantitative yield) of granular white crystals of m.p. 285–295° (discoloration), $\lambda_{max}^{ethanol}$ 239 mμ, λ_{max}^{Nujol} 5.65, 5.99, 6.17, R_{DOC} 1.3 in 10% benzene–hexane. The best samples of this lactone were obtained by sublimation at 230° and 0.1 mm. and crystallization from acetone; they sublimed on the hot-stage without change in appearance at 290–305°.

(B) From Partition Column Side Fractions of Low Activity.—Combined side fractions (377 mg.) from one "first partition"[6] were taken up in 9 ml. of dioxane, 2 g. of periodic acid in 9 ml. of water was added and the cloudy solution allowed to stand at 25° for 1.5 hours. Removal of about half the solvents in a stream of nitrogen was followed by ethyl acetate extraction. This extract was washed with saturated sodium bicarbonate solution. Trituration of the neutral solids with hexane and acetone left 8 mg. of white crystalline solid, which was sublimed *in vacuo* at 240° and the sublimate crystallized from ethyl acetate to yield 5 mg. of white crystalline material of m.p. 295–305°, identical in the infrared with known lactone II.

A 0.25-mg. sample of lactone II was treated for 10 minutes on the steam-cone with acetic anhydride and pyridine; there was obtained a white crystalline residue which melted at 294–296°.

A second 0.25-mg. sample in 0.1 ml. of pyridine was added to the pyridine complex from 2.5 mg. of chromium trioxide. After 16 hours, dilution with water and extraction with ethyl acetate gave white needle-shaped crystals of m.p. 290–305°, which changed to characteristic granular cubes of lactone II in ethyl acetate within 10 minutes.

Lactol-acid III.—Sodium hydroxide solution (2.5 ml. of 2.5 *N*) was added to 5.2 mg. of lactone II in 2.5 ml. of warm methanol. The solution was boiled on the steam-cone for five minutes with loss of most of the methanol and appearance of a yellow color. A trace of crystalline solid was obtained by ethyl acetate extraction of the cooled alkaline solution. Ethyl acetate extraction after acidification followed by decolorization with Norit in acetone gave 5.1 mg. of nearly colorless granular crystals, λ_{max}^{Nujol} 2.9, 3.2, 5.77, 6.00 and 6.18 μ. The transparent cubes became opaque at *ca.* 200° on the micro hot-stage and slow transition to flat blades was observed at *ca.* 220°; melting with discoloration occurred at 285–300°. The lactol-acid did not leave the origin when chromatographed on paper in the benzene system.

A sample of the lactol-acid was sublimed at 230° and 0.1 mm. to yield a white crystalline sublimate identical by infrared spectrum and paper strip mobility with known lactone II.

Lactone II and lactol-acid III were allowed to react with anhydrous 0.05 *N* hydrogen chloride in methanol, prepared from acetyl chloride and dry methanol. The product was a viscous gum, $\lambda_{max}^{CCl_4}$ 5.74 and 5.95 μ, which was not affected by 0.1 *N* aqueous hydrochloric acid, and appeared from infrared examination to be partially converted to lactone II by more drastic acid treatment.

Diazomethane in dioxane was allowed to react with lactol-acid III; there was obtained crystalline methyl ester (IIIa), λ_{max}^{Nujol} 3.01, 5.76, 6.04 and 6.19 μ. Acetic anhydride–pyridine converted this substance to an amorphous acetate, $\lambda_{max}^{CCl_4}$ 5.75, 5.97 and 6.17 μ, from which the methyl ester was regenerated by 0.05 *N* aqueous acid (see "aldosterone acetylation" below).

Diketolactone VIII.—Sodium borohydride (15 mg.) was added to 7.4 mg. of lactol-acid III in 1 ml. of 0.05 *N* sodium hydroxide solution, and the reaction mixture heated on the steam-cone for 1.5 hours. Ethyl acetate extraction after acidification to *p*H 4 gave 7.4 mg. of amorphous white solid polyhydroxy acid VI, λ_{max}^{Nujol} 2.9–3.1 and 5.73–5.85 μ.

Acid VI, 6.6 mg., was heated at 210–220° for 5 minutes in a micro sublimation apparatus; a trace of sublimate was discarded and the non-volatile residue partitioned between ethyl acetate and aqueous sodium bicarbonate to yield 5 mg. of dihydroxylactone VII as a colorless neutral gum, $\lambda_{max}^{CHCl_3}$ 2.9 and 5.72 μ.

Lactone VII in solution in 0.1 ml. of pyridine was added to the complex from 11 mg. of chromium trioxide and 0.2 ml. of pyridine[9] and the mixture kept overnight at room temperature. The reaction mixture, which contained much dark solid, was then brought to *p*H 4 with hydrochloric acid and extracted with ethyl acetate. An ethyl acetate solution of the amber residue was passed through a short column of Norit to yield 3.0 mg. of granular crystalline diketolactone VIII. This compound softened at 211° and melted at 230–237°, λ_{max}^{Nujol} 5.69, 5.89, 6.00 and 6.18 μ.

Quantitative Periodic Acid Oxidation of Aldosterone.—A solution of 4.2 mg. of periodic acid dihydrate in 0.7 ml. of water was added to a solution of 1.002 mg. (2.78 micromoles, assuming mol. wt. 360) of aldosterone in 1 ml. of dioxane and the reaction mixture kept at room temperature for 18 hours. Periodate consumption was determined by the standard arsenite method.[12] Oxidation of the aldosterone consumed 2.68 micromoles (96% of one molecular equivalent) of periodic acid. Titration of a second sample of aldosterone after 25 hours at room temperature indicated no further consumption of oxidant.

Lactone II was extracted from the combined neutral solutions after titration, 6 mg. of dimedone in 0.2 ml. of dioxane added and the solution heated on the steam-cone for 5 minutes. Ethyl acetate extraction gave 2.2 mg. (90% of theoretical for 1 equivalent) of the formaldehyde–dimedone adduct of m.p. 180–190° either alone or mixed with authentic material.

Aldosterone Acetylation.—Boiling acetic anhydride converted crystalline aldosterone in five minutes (80–90% yield) into crystalline 21-monoacetate of m.p. 193–196.5°, $\lambda_{max}^{ethanol}$ 240 mμ (ε, 16400), λ_{max}^{Nujol} 2.85, 5.76, 5.98 and 6.22 μ.

Acetic anhydride–pyridine with either aldosterone or the 21-monoacetate on the steam-cone for 8–10 minutes or overnight at room temperature gave an amorphous gum which contained some 21-monoacetate, R_A 0.83 in 40% benzene–hexane, but was mostly non-crystalline 18,21-diacetate V, R_{DOC} 0.6 in 10% benzene–hexane, $\lambda_{max}^{CCl_4}$ 5.69, 5.76 and 5.94 μ. Monoacetate of m.p. 190–196°, identical by infrared spectrum and paper strip mobility with known aldosterone 21-acetate was obtained in high yield from reaction of the diacetate overnight with aqueous alcohol or dioxane 0.05 *M* in acetic, hydrochloric, or periodic acid.

Hydrolysis of either mono- or diacetate with sodium bicarbonate in aqueous methanol yielded aldosterone, identified by paper strip mobility (R_E 0.9 in the benzene system) and by bio-assay.

Attempted Mesylation of Lactol-acid Methyl Ester IIIa.—A solution of 0.1 mg. of methanesulfonyl chloride in 0.1 ml. of pyridine was added at room temperature to 0.4 mg. of the methyl ester of lactol-acid III and the solution allowed to stand at room temperature for 16 hours. Water (2 ml.) was then added and ethyl acetate extraction carried out. The R_A of the product on paper chromatography in 40% benzene–hexane was 0.84; lactol methyl ester had R_A 0.90.

A second attempt at mesylation with similar quantities of reactants in a sealed capillary tube at 100° for one hour yielded a product which on paper chromatography gave no discernible ultraviolet or tetrazolium-positive spots.

Lactone-acid IV.—A sample of 1.2 mg. of lactol-acid III was oxidized with 1.5 mg. of chromium trioxide in 0.5 ml. of 90% aqueous acetic acid, and the mixture allowed to stand at room temperature overnight. The product was a white crystalline solid which charred slightly but did not melt below 320° and had λ_{max}^{Nujol} 5.64, 5.75, 6.11 μ.

Anal. Calcd. for $C_{20}H_{24}O_5$: O, 23.2; neut. equiv., 345; for $C_{20}H_{22}O_6$: O, 26.8; neut. equiv., 358. Found: O, 23.7; neut. equiv., 354.

A suspension of 0.5 mg. of calcium carbonate and 0.1 mg. of lactol-acid III in 1 ml. of water was shaken at room temperature for five minutes with one drop of bromine. The mixture was then acidified with 6 *N* sulfuric acid and extracted with ethyl acetate. The resulting gum had R_E 1.4 in the benzene system while known lactone-acid IV had R_E 1.3.

Lactone-acid IV (1.4 mg.) was suspended in water and 0.1 *N* sodium hydroxide solution added until all solid material was in solution and the *p*H was about 10. The solution was then freeze-dried; the solid residue had λ_{max}^{Nujol} 5.69, 5.98 and 6.39 μ.

Attempted Opening of Lactone-acid IV.—Lactone-acid IV was treated in a sealed tube at 150° for two hours with 0.1 *N* sodium hydroxide solution; under these conditions, 3-keto-Δ^4-etiocholenic acid was stable. The reaction

(12) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 361.

product, obtained by acidification and extraction, could not be identified positively as starting material but had lactone absorption in the infrared. Lactone absorption was present also in the weak infrared spectra of several samples of the sodium salt of lactone-acid IV, obtained by freeze-drying aqueous solutions of *p*H *ca.* 10.

Lactone-acid was recovered unchanged from solution in ammonia-saturated methanol after 1.5 hours at room temperature, and when its solution in sulfuric acid was quenched in cold methanol. Sealed in a capillary tube at 100° for one hour with 0.5 *N* anhydrous hydrogen chloride in dry methanol, lactone-acid IV yielded a crystalline product of m.p. 204–213° with softening at 190°. This material, probably the methyl ester, was insoluble in 1 *N* sodium hydroxide and had λ_{max}^{Nujol} 5.66, 5.79, 6.0 and 6.19 μ.

Acknowledgments.—We wish to thank Mrs. E. V. Hagan for competent technical assistance. We are indebted to Mr. R. N. Boos and associates for the microanalyses and to Dr. Herbert Stoerk and Dr. David Tennent and their co-workers, at the Merck Institute for Therapeutic Research, for the bioassays. We acknowledge the services of Mr. Robert Walker who obtained the infrared data and Mrs. Helen Gager who made the potentiometric measurements. Thanks are due Dr. Karl Folkers for advice and encouragement.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Some Salts of the Phosphoric Ester of Vitamin D_3

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RECEIVED OCTOBER 5, 1954

A new phosphorylation method applicable to very sensitive substances like vitamins A and D has been developed. This method has been applied to vitamin D_3 and the sodium, calcium and barium salts of vitamin D_3 phosphate have been obtained in micro-crystalline form. While the sodium salt is completely soluble in water the others are insoluble, but dissolve readily in hydrocarbon solvents. From their physical properties these salts have been found to be polymeric (trimeric) and tentative structures have been suggested for each.

The phosphorylation of vitamin D_2 (calciferol) and vitamin D_3 (from tuna fish liver oil) was first reported by one[2] of us who also prepared water-soluble salts of the phosphoric acid esters of these vitamins. The sodium salt of vitamin D_2 phosphate also was prepared recently by Zetterström[3,4] who found that it doubles the enzyme activity of alkaline kidney phosphatase at the beginning of the incubation period as compared to the activity of this enzyme in the absence of the vitamin. Unphosphorylated vitamin D_2 suspended in water had no influence on the activity of alkaline kidney phosphatase. However, no attempt was made either by Milas or by Zetterström, *et al.*, to purify the sodium salt or any other salt of vitamin D phosphate. We therefore wish to report the preparation, purification and determination of physical properties of four different salts of vitamin D_3 and to propose a tentative structure of the same.

The pyridine method[2–4] which was used by the early workers was found to give low yields and the product obtained was difficult to purify, owing perhaps to the dehydrating action of phosphorus oxychloride[5–7] which led to undesirable by-products. Attempts subsequently to phosphorylate vitamin D_3 with diphenyl chlorophosphate[8,9] and removing the protecting groups led to the destruction of most of the vitamin. We therefore resorted to one of the original methods[2] using instead of sodium triphenylmethyl, phenyllithium to prepare the lithium vitaminate which was allowed to react with phosphorus oxychloride to form vitamin D_3 dichlorophosphate. To obtain the calcium salt, the vitamin D_3 dichlorophosphate was hydrolyzed with an aqueous suspension of calcium hydroxide. One of the barium salts also was made by hydrolysis of the dichloride with an aqueous solution of barium hydroxide. Both the calcium and the barium salts are soluble in hydrocarbon solvents and are obtained as white micro-crystalline solids. The yields of these salts were not entirely satisfactory and it was not possible to obtain the pure sodium salt by this method. An attempt to obtain the pure ester or the pure sodium salt by treating the calcium salt with aqueous oxalic acid or sodium oxalate or citrate failed to remove the calcium, and the original salt was recovered unchanged even after prolonged contact.

A more general method which also is applicable to other sensitive biological products like vitamin A[10] consists of allowing the lithium derivative of vitamin D_3 to react in an inert solvent and in an atmosphere of pure nitrogen with di-*t*-butyl chlorophosphate made *in situ* by treating at low temperatures phosphorus oxychloride with two mole-equivalents of pure solid lithium *t*-butoxide. The di-*t*-butyl vitamin D_3 phosphate thus formed is hydrolyzed readily with either trisodium phosphate or a suspension of calcium hydroxide to form in good yields the corresponding sodium and calcium salts of vitamin D_3 phosphate. The calcium salt produced by this method is identical with that as made by the previous method. However, a barium salt made from the purified sodium salt was not the same as that produced by the first method. Table I records some of the physical properties and analytical data of these salts.

(1) Research Associate.

(2) N. A. Milas, U. S. Patent 2,296,291 (Sept. 22, 1942).

(3) R. Zetterström, *Nature*, **167**, 409 (1951).

(4) R. Zetterström and M. Ljunggren, *Acta Chem. Scand.*, **5**, 283 (1952).

(5) E. Seebeck and T. Reichstein, *Helv. Chim. Acta*, **26**, 536 (1943); H. Reich and T. Reichstein, *ibid.*, **26**, 562 (1943).

(6) C. Djerassi, E. Batres, M. Velasco and G. Rosenkranz, THIS JOURNAL, **74**, 1712 (1952).

(7) G. Rosenkranz, O. Mancera and F. Sondheimer, *ibid.*, **76**, 2227 (1954).

(8) H. Bredereck, E. Berger and J. Ehrenberg, *Ber.*, **73**, 269 (1940).

(9) A. R. Todd, *J. Chem. Soc.*, 647 (1946).

(10) Results on salts of vitamin A phosphate will be published in a subsequent article.