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.

RAHWAY, N. J.

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

Some Salts of the Phosphoric Ester of Vitamin D_3

BY NICHOLAS A. MILAS, PAULS DAVIS[1] AND LI-CHIN CHIANG[1]

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.

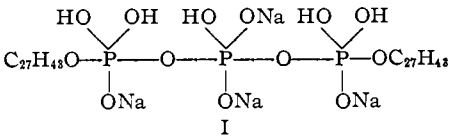
TABLE I

PHYSICAL PROPERTIES OF SOME SALTS OF VITAMIN D_3 PHOSPHATE

Salt	M.p., °C.	$E_{1cm}^{1\%}$ [λmax (mμ)]	Formula	Calcd. P, %	Calcd. Metal, %	Calcd. Mol. wt.	Found P, %	Found Metal, %	Found Mol. wt.
Na (I)	215–216 dec.	207 (265–266) water	$C_{54}H_{91}P_3O_{13}Na_4$	8.20	8.12	1133	8.15	8.09	1082
(II)	.....	.....	$C_{54}H_{89}P_3O_{12}Na_4$	8.34	8.25	1115			
Ca (III)	210–211 dec.	242 (264.5) cyclohexane	$C_{81}H_{131}P_3O_{13}Ca_3$	6.10	7.87	1526	6.28	8.10	1455
(IV)	.....	.....	$C_{81}H_{129}P_3O_{12}Ca_3$	6.20	8.00	1508			
Ba (V)	193–194 dec.	178 (267) cyclohexane	$C_{81}H_{131}P_3O_{12}Ba_2$	5.58	16.52	1664	5.25	17.36	1581
(VI)	.....	.....	Same	5.58	16.52	1664			
(VII)	175–180 dec.	.....	$C_{54}H_{92}P_2O_{10}Ba$	5.63	12.47	1101	5.50	12.20	..

Discussion of Results

It is quite obvious from the results recorded in Table I that the salts of vitamin D_3 phosphate are polymeric. This is not at all surprising since the hydrolysis of the triesters of phosphoric acid[11] lead frequently to derivatives of pyrophosphoric acid. Furthermore, Wagner-Jauregg, *et al.*,[12] have shown that on treatment of the monocholesteryl ester of phosphoric acid with basic solutions, the product recovered was dimeric and a derivative of pyrophosphoric acid. Similar results were reported by Friedman and Seligman[13] with the mononaphthyl ester of phosphoric acid.



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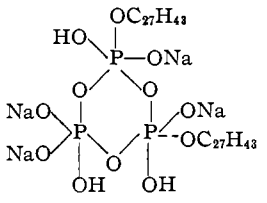

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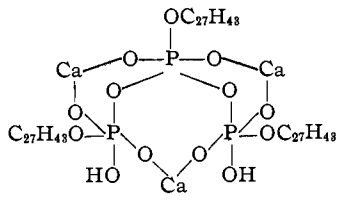

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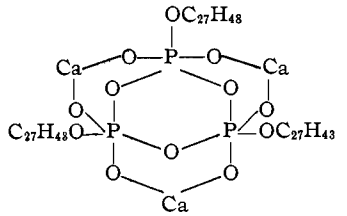

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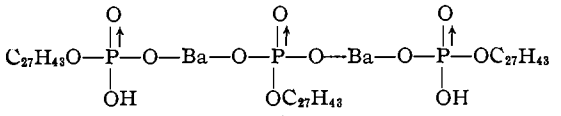

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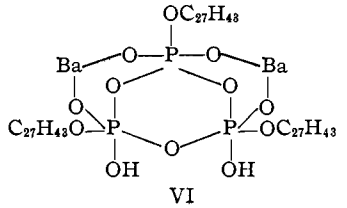


VI

Of all the structures considered for the three salts of vitamin D_3 phosphate for which molecular weights and spectroscopic data are available, structures I–VI seem most reasonable and a decision between the members of each pair was sought in the interpretation of their infrared spectra. The infrared spectra of these salts were determined by the pellet method using 1% of each salt in potassium bromide, and by the mulling method in Nujol. Both methods gave essentially the same results. The spectra were recorded by the Baird double beam infrared recording spectrophotometer, model B. The characteristic bands of the infrared spectra of these salts are listed in Table II.

TABLE II

CHARACTERISTIC MAXIMA OF INFRARED BANDS OF CERTAIN VITAMIN D_3 SALTS

Salt	Assignment of characteristic frequencies, cm.$^{-1}$ P–O–P	P–O	P–O–C	P=O (bonded)
Na	720m	978bs	1020–1120bs	1240w
Ca	722m	990bs	1030–1110bs	1210m
Ba	720m	978bs	1020–1110bs	1210w

w = weak, m = medium, bs = broad, strong

The shallow band at 720–722 cm.$^{-1}$ is in the region attributed to the P–O stretching of the P–O–P group by Holmstedt and Larson.[14] The strong band at 978–990 cm.$^{-1}$ is very close to the region of 930–950 cm.$^{-1}$ which was assigned by Bergmann, *et al.*,[15] to the P–O–P band. However, in a more recent article Bellamy and Beecher[16] ascribe the 980 cm.$^{-1}$ band to the P–O stretching mode of the pentavalent phosphorus atom. The band in the region of 1020–1100 cm.$^{-1}$ with a center at 1050 cm.$^{-1}$ has been assigned by several investiga-

(11) E. Cherbuliez and J.-P. Leber, *Helv. Chim. Acta*, **35**, 2592 (1952).

(12) T. Wagner-Jauregg, T. Lennartz and H. Kothny, *Ber.*, **74**, 1513 (1941); T. Wagner-Jauregg and T. Lennartz, *ibid.*, **75**, 178 (1942); T. Wagner-Jauregg and A. Wildermuth, *ibid.*, **77**, 481 (1944).

(13) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **73**, 5292 (1951).

(14) B. Holmstedt and L. Larson, *Acta Chem. Scand.*, **5**, 1179 (1951).

(15) E. D. Bergmann, U. Z. Littauer and S. Pinchas, *J. Chem. Soc.* 847 (1952).

(16) L. J. Bellamy and L. Beecher, *ibid.*, 728 (1953).

tors[16-18] as the characteristic band for the P–O–C linkage. This is also the region attributed to the P–OH deformation vibration,[16] but it is usually masked by the strong P–O–C absorption.

The P=O (unbonded) band at 1250–1300 cm.$^{-1}$ which occurs in nearly all the esters of phosphoric acid is absent in the infrared spectra of the salts of vitamin D_3 phosphate. However, a medium to weak band is present at 1210–1240 cm.$^{-1}$ which is probably due to hydrogen bonded P=O groups.[16] The absence of a band at 2560–2700, usually attributed to the P–OH stretching, is not surprising since salts of phosphoric acid esters show no absorption in this region.

On the basis of these results and those shown in Table I, it is reasonable to propose that the sodium salt has either the cyclic structure II or the linear structure I while the calcium and barium salts probably have structures IV and VI, respectively.

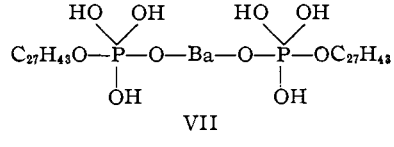
The sodium salt of vitamin D_3 phosphate in deoxygenated aqueous solutions is labile as shown by the ultraviolet absorption measurements of these solutions listed in Table III. The shift of the ultraviolet absorption maximum from 265–266 mμ to 251–252 mμ after 20.5 hours of standing under nitrogen at room temperature was reversed to 261–264 mμ upon regeneration of the acid ester by acidification with dilute phosphoric acid. The sodium salt does not show this property in non-aqueous solvents. It is therefore possible that under the influence of hydroxide ions (*p*H of the solution was 7.5–8) the vitamin D_3 portion of the sodium salt slowly undergoes isomerization of the *cis–trans* type.[19]

TABLE III

THE ULTRAVIOLET ABSORPTION SPECTRA OF DEOXYGENATED AQUEOUS SOLUTIONS OF THE SODIUM SALT OF VITAMIN D_3 PHOSPHATE

Time, min.	13	73	143	193	1230
λ_{max}, mμ	265–266	264–265	259–264	259–261	251–252
$E^{1\%}_{1\ cm.}$	207	183	165.5	162	133

In the absence of molecular weight and infrared spectrum data for the monobarium salt we wish to propose structure VII which seems to agree closely



VII

with the analytical data. This is analogous to the monosodium salt of cholesteryl dihydrogen phosphate reported by other investigators.[12,13]

Experimental[20]

Phenyllithium.—This was prepared according to Jones and Gilman[21] and standardized by titration against standard hydrochloric acid using phenophthalein as indicator.

Lithium *t*-Butoxide.—This was prepared by adding small pieces of metallic lithium to a large excess of freshly distilled (from calcium hydride) *t*-butyl alcohol. The excess alcohol was then removed under a reduced pressure, the white solid residue dissolved in anhydrous pentane and the mixture filtered in nitrogen through a sintered glass plate of coarse porosity. The solvent was again removed under a reduced pressure and the white solid analyzed by titration with standard hydrochloric acid. It was found to be pure lithium *t*-butoxide.

Calcium Vitamin D_3 Phosphate (from Vitamin D_3 Dichlorophosphate).—To a cold (0°) solution of anhydrous ether (30 cc.) containing 1.012 g. of crystalline vitamin D_3[22] was added in nitrogen with stirring 2.63 cc. of 1 *N* ethereal solution of phenyllithium. After one hour of standing at room temperature the mixture was cooled to 0° and to it was added with stirring 0.4033 g. of phosphorus oxychloride in about 10 cc. of anhydrous ether and the resulting mixture allowed to stand at 0° for two hours longer. The ethereal solution was then decanted into a Thiele tube, mixed with 60 cc. of 0.2 *N* calcium hydroxide suspension, the tube deoxygenated, sealed in nitrogen and shaken in the dark for 24 hours.

The ether and most of the water were then removed in nitrogen at room temperature and under a reduced pressure. In this operation the water vapor carried with it traces of diphenyl which is always produced in the preparation of phenyllithium. The residue was taken up in ether, the ethereal solution filtered through a sintered glass funnel, and the ether removed in vacuum. The residue was dried azeotropically by dissolving it several times in pure benzene and removing the latter each time in vacuum. It was finally obtained as a white powder which was recrystallized several times from absolute ethanol and dried for several hours under a high vacuum in a pistol containing phosphorus pentoxide; yield 0.6303 g. (47%), m.p. 210–211° dec.

Anal. Calcd. for $C_{81}H_{129}P_3O_{12}Ca_3$: P, 6.20; Ca, 8.00; mol. wt., 1508. $C_{81}H_{131}P_3O_{13}Ca_3$: P, 6.10; Ca, 7.87; mol. wt., 1526. Found: P, 6.28; Ca, 8.10; mol. wt. by the Rast method using "exaltone," 1455.

In camphor which has a much higher m.p. than "exaltone" this salt showed considerable decomposition and the mol. wt. was not reproducible; inorganic residue found by combustion, 25.7%; calcd., 25.2%. The extinction coefficient ($E^{1\%}_{1cm.}$) at 264.5 mμ cyclohexane was found to be 242, and the characteristic bands of the infrared spectrum are listed in Table II.

This salt is soluble in all common hydrocarbon solvents and slightly soluble in methanol and ethanol. It is insoluble in water.

Calcium Vitamin D_3 Phosphate (from Di-*t*-butyl Vitamin D_3 Phosphate).—To a cold (0°) solution of dry petroleum ether (50 cc.) in a Thiele tube containing 0.24 cc. (0.0026 mole) of phosphorus oxychloride was added dropwise with shaking a cold (0°) solution of petroleum ether (50 cc.) containing 0.4164 g. (2 × 0.0026 mole) of lithium *t*-butoxide. A white precipitate appeared at once. The mixture (marked I) was then allowed to stand at 0° for three hours longer. The lithium derivative of vitamin D_3 was made as follows: to a cold (0°) solution of anhydrous ether (20 cc.) containing 1.0 g. of vitamin D_3 in another Thiele tube was added, with shaking in an atmosphere of nitrogen, 11.29 cc. (0.0026 mole) of 0.2304 *N* ethereal solution of phenyllithium, and the mixture (marked II) was allowed to stand at 0° for one hour. II was then slowly added to I with shaking and the resulting product allowed to stand in the refrigerator overnight. An equal volume of water was then added and the mixture subjected at room temperature to a vacuum distillation to remove the solvent and the water. The yellowish waxy residue was redissolved in ether and the ethereal solution thoroughly shaken with 1% phosphoric acid, then with water. The ether layer was transferred into a Thiele tube and shaken for 24 hours in nitrogen and in the dark with a suspension of 4 g. of calcium hydroxide in 50 cc. of water. The calcium salt was obtained as in the previous case and dried azeotropically using benzene; yield of the crude product, 1.3 g. This was purified further by triturating it several times with absolute ethanol and drying it

(17) C. Meyrick and H. Thompson, *J. Chem. Soc.*, 225 (1950).

(18) L. W. Daasch and D. C. Smith, *Anal. Chem.*, **23**, 853 (1951).

(19) H. H. Inhoffen and K. Brückner, *Fortsch. Chem. Org. Naturstof.*, **11**, 83 (1954).

(20) All m.p.s. are uncorrected. All experiments reported in this investigation were carried out under prepurified nitrogen.

(21) R. G. Jones and H. Gilman, *Org. Reactions*, **6**, 355 (1951).

(22) The crystalline vitamin D_3 used in this investigation was from two sources; one purchased from du Pont Co., the other was kindly supplied to us by Vitamins, Inc. Both samples had essentially the same spectroscopic properties.

for several hours under high vacuum over phosphorus pentoxide: yield 1 g. (76.5%), m.p. 210–211° dec.

Sodium Vitamin D_3 Phosphate (from Di-*t*-butyl Vitamin D_3 Phosphate).—Since it was not possible to obtain the pure sodium salt from vitamin D_3 dichlorophosphate, it was obtained from di-*t*-butyl vitamin D_3 phosphate. An ethereal solution of vitamin D_3 dihydrogen phosphate (from 1 g. of vitamin D_3) prepared as in the previous case was placed in a Thiele tube and shaken for 24 hours in the dark and in an atmosphere of nitrogen with a solution (50 cc.) of trisodium phosphate dodecahydrate (2 g.). The ether layer was then separated and the aqueous layer extracted several times with small portions of benzene. The benzene extracts were combined with the ether layer and the mixture concentrated to a small volume at room temperature and under reduced pressure. The concentrate, which was very cloudy, was filtered through a sintered glass funnel and the filtrate evaporated to dryness in vacuum; yield 1.3 g. This was further purified by triturating it with absolute ethanol, and the mixture filtered. The yellowish solid obtained was dissolved in anhydrous ether, the mixture filtered again and the ether removed in vacuum. The white solid residue was washed once with absolute ethanol and dried for 12 hours under a high vacuum in the presence of phosphorus pentoxide; yield 410 mg. (32.3%), m.p. 215–216° dec.

Anal. Calcd. for $C_{54}H_{91}P_3O_{13}Na_4$: P, 8.20; Na, 8.12; mol. wt., 1133. $C_{54}H_{89}P_3O_{12}Na_4$: P, 8.34; Na, 8.25; mol. wt., 1115. Found: P, 8.15; Na, 8.09; mol. wt. (in "exaltone"), 1082.

The sodium in the sodium vitamin D_3 phosphate was determined in water solution by flame photometry.[23]

The ultraviolet spectrum of this salt in water is shown in Table III, and the characteristic bands of its infrared spectrum are listed in Table II.

This salt is completely soluble in water, forming clear solutions; it is soluble in benzene, tetrahydrofuran and ether, difficultly soluble in alcohol, insoluble in aliphatic hydrocarbon solvents.

Barium Vitamin D_3 Phosphate (from the Sodium Salt).—A solution of sodium vitamin D_3 phosphate in ether was shaken with 5% phosphoric acid solution, then with water. It was then transferred into a Thiele tube and shaken for 24 hours in the dark and in nitrogen with an excess solution of 0.1 *N* barium hydroxide. The ether layer was then removed and the aqueous layer extracted several times with ether and the ether extracts combined and washed once with water. The ether was then removed in vacuum and the residue dried azeotropically with benzene. The light brown solid obtained was further purified by triturating with absolute alcohol and the mixture filtered. The solid obtained was dried for several hours under high vacuum and in the presence of phosphorus pentoxide; m.p. 193–194° dec.

Anal. Calcd. for $C_{81}H_{131}P_3O_{12}Ba_2$: P, 5.58; Ba, 16.52; mol. wt., 1664. Found: P, 5.25; Ba, 17.36; mol. wt. (in "exaltone"), 1581.

The extinction coefficient ($E_{1\,cm.}^{1\%}$) at 267 mμ in cyclohexane was found to be 178, and the characteristic bands of its infrared spectrum are listed in Table II.

The barium salt is insoluble in water, soluble in hydrocarbon solvents, slightly soluble in ethanol.

Barium Vitamin D_3 Phosphate (from Vitamin D_3 Dichlorophosphate).—This salt was prepared and purified according to the procedure given for the calcium salt (from vitamin D_3 dichlorophosphate) except the hydrolysis of vitamin D_3 dichlorophosphate which was accomplished with 0.1 *N* barium hydroxide solution. From 769.2 mg. of vitamin D_3 was obtained 1.065 g. (96%) of the purified barium salt, m.p. 175–180° dec. This salt is soluble in all hydrocarbon solvents, slightly soluble in ethanol, insoluble in water.

Anal. Calcd. for $C_{54}H_{92}P_2O_{10}Ba$: P, 5.63; Ba, 12.47; inorganic residue, 27.5. Found: P, 5.50; Ba, 12.20; inorganic residue found by combustion, 27.1.

Acknowledgment.—The authors wish to acknowledge with thanks some of the financial support for this investigation from the Research Corporation. They also wish to thank Dr. Nagy and his associates of this Institute for some of the analyses and the infrared spectra.

CAMBRIDGE 39, MASSACHUSETTS

(23) J. U. White, *Anal. Chem.*, **24**, 394 (1952).

[CONTRIBUTION NO. 1214 FROM STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

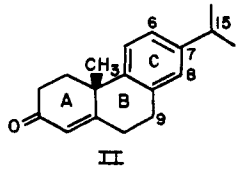
Chromic Acid Degradation of Methyl Dinitrodehydroabietate[1]

BY ERIK S. HANSEN AND HAROLD H. ZEISS[2]

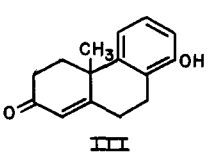
RECEIVED JANUARY 27, 1954

Chromic acid oxidation of methyl dinitrodehydroabietate leads to extensive rupture of the hydrophenanthrene ring system. The degradation products are characterized and assigned structures.

Stimulated by the successful conversion of dehydroabietic acid (I) into 2-keto-$\Delta^{1,11}$-nordehydroabietene (II) and by an encouraging stereochemical relationship of the C-12 methyl group,[3] our interest in the investigation of the diterpenic acids as source material for steroidal syntheses turned to the final obstacle, the modification of ring C. Starting with any of the abietic-type acids involves the task of removing the C-7 isopropyl group, while the use of *d*-pimaric acid leads to another set of difficulties. The intermediate III, obtained by Robinson and Cornforth[4] in their total synthesis of steroids, seemed to us to be the most suitable compound with which to establish rapport, both structural and stereochemical, between dehydroabietic acid and the steroids. The problem thus proposed was desisopropylation at C-7 and appropriate substitution at C-8.



II



III

Other workers have subjected I to various oxidative conditions. Drake has oxidized I with oxygen and persulfate catalyst in alkaline solution to 9-ketodehydroabietic acid.[5] The same keto acid was obtained from I by the action of alkaline permanganate by Pratt.[6] Air oxidation of methyl dehydroabietate in the presence of benzoyl peroxide by

(1) Taken from the dissertation submitted by E. S. Hansen to the Faculty of the Graduate School, Yale University, 1953, in candidacy for the Ph.D. degree.

(2) To whom correspondence may be addressed.

(3) H. H. Zeiss and W. B. Martin, Jr., THIS JOURNAL, **75**, 5935 (1953).

(4) R. Robinson and J. W. Cornforth, *J. Chem. Soc.*, 1855 (1949).

(5) A. E. Drake, U. S. Patent 2,434,643; *C. A.*, **42**, 2786*d* (1948).

(6) Y. T. Pratt, THIS JOURNAL, **73**, 3803 (1951).