

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 83.61; H, 10.03.
Found: C, 83.43; H, 10.02.

7-Dehydrocampesterol.—A solution of 150 mg. of dehydrocampesteryl benzoate in 5 cc. of benzene was added to 10 cc. of 5% methanolic potassium hydroxide. After boiling for two hours, the hydrolyzed product was obtained by ether extraction and crystallized from acetone-methanol. 7-Dehydrocampesterol formed shining irregular plates, m. p. 164–165°, $[\alpha]_D^{25} -109.0^\circ$ (0.96% in chloroform). The sample for analysis was dried for two and one-half hours at 107° in a high vacuum.

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63.
Found: C, 84.81; H, 11.58.

The absorption spectrum in ether exhibited the maxima at 272 $m\mu$ and 282 $m\mu$ characteristic of 7-dehydrosterols; $\epsilon_{282\ m\mu} = 10,600$.

Irradiation with Ultraviolet Light.—The light source used in this work was a 125-watt air-cooled quartz mercury vapor lamp. For each run 63 cc. of a 0.1% solution of the sterol in peroxide-free ether was taken, and after displacing the dissolved air with a stream of carbon dioxide, irradiation commenced with the preheated lamp. After irradiation the solution was evaporated to dryness, taken up in a few drops of alcohol and then made up to 6.3 cc. with corn

oil for assay. Preliminary experiments with ergosterol showed that four minutes was the optimal time for irradiation. Under these conditions ergosterol gave a product the activity of which assayed by the U. S. P. XI line test on rats was found to be 7,000,000 international units of vitamin D per gram of original ergosterol. This corresponds to a conversion of 17.5%. 7-Dehydrocampesterol irradiated under identical conditions assayed 725,000 international units per gram of original substance.¹³

Summary

The preparation and properties of 7-dehydrocampesterol are described. 7-Dehydrocampesterol on irradiation with ultraviolet light yields an antirachitically active product. By comparison with ergosterol irradiated under the same conditions the antirachitic potency of the vitamin derived from 7-dehydrocampesterol has been estimated as 4,100,000 international units per gram.

(13) Preliminary results indicated that the vitamin from 7-dehydrocampesterol resembles vitamin D₂ rather than vitamin D₃ in that it is relatively less active when assayed by the chick test.

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[CONTRIBUTION FROM THE SHELLAC RESEARCH BUREAU OF THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Nature and Constitution of Shellac. XVI. Preparation of 8,9,15-Trihydroxypentadecylamine from Aleuritic Acid by the Naegeli-Curtius Series of Reactions¹

BY ARTHUR L. DAVIS² AND WM. HOWLETT GARDNER

Introduction

Aliphatic polyhydroxyamines have many interesting properties.³ They should be valuable intermediates in the synthesis of several new compounds which would be useful in the paint and other fields. Such hydroxyamines might be prepared from polyhydroxy acids which have been obtained from shellac.^{4,5} The hydroxyl groups of such acids, however, have a tendency to react intermolecularly when subjected to elevated temperatures of 100° or higher, such as are employed in a number of common methods for obtaining amines from carboxylic acids. This was what apparently occurred in attempts to prepare the

amide when using the Hofmann procedure.⁵ The Lossen method involving the formation of a substituted hydroxamic acid also gives very poor results. The Curtius method⁶ likewise has proved unsatisfactory. Nagel obtained only a mixture of partially chlorinated amines when he attempted to prepare 8,9,15-trihydroxypentadecylamine from aleuritic acid by this method. His failure can be traced to the use of concentrated hydrochloric acid in hydrolyzing the relatively stable trihydroxypentadecylurethan. Other strong mineral acids lead to like difficulties.⁶ Naegeli⁷ had similar trouble in attempting to synthesize the amine from chaulmoogric acid. Hence he was led to prepare the isocyanate from the azide instead of the urethan. Isocyanates generally can be hydrolyzed to the amines in the presence of aqueous solutions of alkali without affecting the hydroxyl groups. These series of reactions can be expressed as follows wherein the group $C_{15}H_{31}O_8$ may be rep-

(1) This communication is part of a thesis for the degree of Master of Science in Chemistry presented by Arthur L. Davis to the Graduate Faculty of Polytechnic Institute of Brooklyn, June, 1941.

(2) Shellac Research Fellow, 1939–1942.

(3) (a) B. M. Vanderbilt and H. B. Hass, *Ind. Eng. Chem.*, **32**, 35–36 (1940); (b) M. M. Sprung, *This Journal*, **61**, 3381 (1939).

(4) (a) B. B. Schaeffer and W. H. Gardner, *Ind. Eng. Chem.*, **30**, 333 (1938); (b) H. Weinberger and W. H. Gardner, *ibid.*, **30**, 454 (1938); (c) P. M. Kirk, P. Spoerri and W. H. Gardner, *This Journal*, **63**, 1243 (1941).

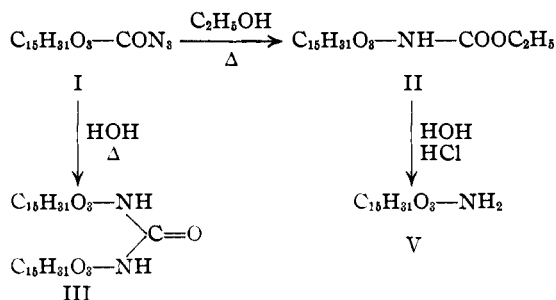
(5) A. L. Davis, Master's Dissertation, Polytechnic Inst. of Brooklyn, Brooklyn, N. Y., June, 1941.

(6) Th. Curtius, *Ber.*, **27**, 779 (1894).

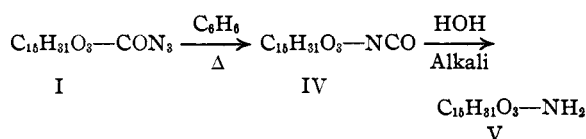
(7) C. Naegeli, I. Grüntuch and P. Lendorff, *Helv. Chim. Acta* **12**, 227 (1929).

resented as, $\text{CH}_2\text{OH}-(\text{CH}_2)_5-\text{CHOH}-\text{CHOH}-(\text{CH}_2)_7-$.

CURTIUS SERIES OF REACTIONS



NAEGELI MODIFICATION



This article describes how 8,9,15-trihydroxypentadecylamine was prepared from aleuritic acid by such a method. Kirk has already suggested how such a procedure might be employed in the study of shellac acids of unknown structure, such as shellolic acid.^{4c,8} This study of shellolic acid will be described in a later communication.

In an attempt to prepare the amine corresponding to aleuritic acid (9,10,16-trihydroxypalmitic acid) with the aid of the Curtius series of degradation reactions, the acid was esterified directly with anhydrous methyl alcoholic hydrogen chloride. The product, methyl aleuritate, was converted to the acid hydrazide, by the action of hydrazine hydrate on an alcoholic solution of the ester. This conversion was practically quantitative when highly purified samples of the ester were used. Nitrosation, at temperatures approaching 0°, converted the hydrazide into aleurityl azide I, an unstable, hygroscopic solid. When I was heated in water for three hours, N,N'-bis-8,9,15-trihydroxypentadecyl urea III was formed, while when I was refluxed with anhydrous ethyl alcohol for one and one-half hours, 8,9,15-trihydroxypentadecylurethan II was the resulting product. Inasmuch as hydrolytic action failed to convert either II or III into the corresponding amine, the Naegeli modification was resorted to. When I was decomposed by heating in anhydrous benzene for one hour, the corresponding isocyanate was formed.

(8) P. M. Kirk, Doctor's dissertation, Polytechnic Institute of Brooklyn, Brooklyn, N. Y., June, 1939.

Pure 8,9,15-trihydroxypentadecyl isocyanate IV which was prepared for the first time, was a colorless, crystalline solid melting at 103.5–104.5°. It was soluble in alcohol and dioxane but practically insoluble in diethyl ether, petroleum ether and water. The isocyanate reacted with water and alcohol to produce N,N'-bis-8,9,15-trihydroxypentadecylurea III and 8,9,15-trihydroxypentadecylurethan II, respectively. These compounds were identical with those obtained previously by Nagel^{9,10} from aleurityl azide except that the urethan was more highly purified.

Hydrolysis of the isocyanate IV with hot aqueous alkali produced 8,9,15-trihydroxypentadecylamine V which was obtained as minute colorless crystals melting at 146–147°. This product was soluble in aqueous solutions of mineral acids and gave the characteristic qualitative tests for primary aliphatic amines.¹¹ The picrate of the amine was a yellow powder melting at 118–119°.

Experimental Part

Aleuritic Acid.—A suspension of 100 g. of crude zinc salt in a liter of hot (90°) 20% sulfuric acid was stirred mechanically for thirty minutes, filtered and cooled quickly with ice cubes and the precipitated acid removed by filtration. This was dissolved in ethanol, decolorized with Norit, repeated if necessary, precipitated with water, recrystallized from hot water and dried at 41°; m. p. 101–101.5°. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{32}\text{O}_8$: C, 63.15; H, 10.53. Found: C, 63.09, 63.27; H, 10.98, 10.79.

Methyl Aleuritate.—A solution of 10 g. of aleuritic acid in 80 ml. of 5% hydrogen chloride in absolute methanol, was allowed to stand for three days, then was neutralized with a solution of sodium hydroxide in absolute methanol, the precipitated sodium chloride filtered off and the filtrate poured into a large excess of cold (10°) water. The solid was filtered, washed with 10% aqueous sodium carbonate, stirred for thirty minutes, filtered, the precipitate washed with water, dried on a porous plate, and recrystallized from alcohol, yield 86.5%; m. p. 73°. *Anal.* Calcd. for $\text{C}_{17}\text{H}_{34}\text{O}_8$: C, 64.15; H, 10.69. Found: C, 63.99, 64.07; H, 10.81, 10.90.

Aleurityl Hydrazide.—A solution of 10 g. of highly purified methyl aleuritate in 100 ml. of 30% hydrazine hydrate in methanol was refluxed for forty minutes on a water-bath and then filtered through fluted paper, while still hot, to remove any insoluble impurities. The solution, in cooling, deposited crystals of the hydrazide, which were removed by means of a Büchner funnel with the aid of partial vacuum. The filtrate was then concentrated under reduced pressure so as to yield more crude aleurityl hydrazide, which was recrystallized thrice from hot (90°) water, yield 98%; m. p. 139–139.5°. *Anal.* Calcd. for

(9) W. Nagel, *Ber.*, **60**, 805 (1927).

(10) W. Nagel, *Wiss. Veroffenl. Siemens-Konzern*, **10**, 108 (1931).

(11) O. Kamm, "Qualitative Organic Analysis," John Wiley and Sons, New York, N. Y., 2nd ed., 1932, p. 158.

$C_{18}H_{34}O_4N_2$: C, 60.38; H, 10.69; N, 8.81. Found: C, 60.16, 60.40; H, 10.95, 10.61; N, 8.81, 8.88.

Aleurityl Azide.—A solution of 3 g. of pure aleurityl hydrazide in 600 ml. of hot (90°) water was carefully and slowly cooled to below 5° to produce supercooling. Despite the low solubility of the hydrazide in water, no precipitate formed under these conditions.

51 ml. of an aqueous solution (2%) of sodium nitrite at 0° was cautiously added with gentle stirring, before acidification of the system with 12 ml. of an iced (0°) 25% solution of acetic acid. On stirring, the white flocculent azide separated spontaneously.

The azide was filtered with the aid of gentle suction, washed with water and, finally, dried in an evacuated vacuum desiccator containing solid sodium hydroxide which was maintained at 10° , by placing in a refrigerator, yield 90%; decomp. 52° . The azide was soluble in chloroform and ethyl alcohol, partially soluble in ether and benzene, and completely insoluble in water and petroleum ether. It reacted rapidly with hot water (60°) evolving nitrogen, while a similar decomposition occurred when it was heated on a porcelain spatula. It did not explode when struck sharply with a hammer, on an iron plate.

8,9,15-Trihydroxypentadecyl Isocyanate.—A suspension of 3 g. of the azide in 150 ml. of anhydrous benzene was heated slowly under a reflux condenser for one hour. When decomposition of the azide had been completed and a clear solution remained, the insolubles were removed by filtration and the solution allowed to come to room temperature in a desiccator. Sufficient anhydrous petroleum ether (b. p. $28-38^\circ$) was added to the cold filtrate to precipitate the isocyanate completely. The coagulated precipitate was filtered, washed, dried on an unglazed plate and recrystallized from anhydrous dioxane, yield 92%; m. p. $103.5-104.5^\circ$. *Anal.* Calcd. for $C_{18}H_{34}O_4N$: C, 63.78; H, 10.29; N, 4.65. Found: C, 63.65, 63.91; H, 10.37, 10.59; N, 4.30, 4.42. The pure isocyanate was soluble in alcohol and dioxane, but insoluble in ether, petroleum ether, and water.

N,N'-Bis-8,9,15-trihydroxypentadecyl Urea. Method I.—A suspension of 3 g. of pure aleurityl azide in 200 ml. of water was refluxed for three hours over a small flame. When cool, the urea was filtered from the solution, dried on an unglazed plate and recrystallized from ethyl alcohol; m. p. $122.5-123^\circ$. *Anal.* Calcd. for $C_{31}H_{64}O_7N_2$: C, 64.58; H, 11.11; N, 4.86. Found: C, 64.50, 64.38; H, 11.27, 11.19; N, 4.73, 4.79. **Method II.**—A suspension of 0.2 g. of pure 8,9,15-trihydroxypentadecylisocyanate in 100 ml. of water was refluxed, at 100° for three hours. The system was cooled, the precipitate filtered, dried on an unglazed plate and recrystallized from ethyl alcohol; m. p. $122.5-123^\circ$.

Mixtures of various proportions of this product with that obtained by Method I, showed no lowering of the melting point. It was only slightly soluble in cold ethyl alcohol, but dissolved easily at temperatures approaching the boiling point.

8,9,15-Trihydroxypentadecylurethane. Method I.—A solution of 3 g. of pure aleurityl azide in 60 ml. of absolute ethyl alcohol was refluxed for one hour on a water-bath. The resulting solution, when cold, was filtered, added to an excess of anhydrous diethyl ether and the solid filtered,

dried and recrystallized thrice from hot anhydrous ethyl acetate; m. p. $78-79^\circ$. *Anal.* Calcd. for $C_{18}H_{37}O_6N$: C, 62.25; H, 10.66; N, 5.03. Found: C, 62.37, 62.39; H, 10.89, 10.79; N, 5.03, 5.06. The pure product melted sharply at $78-79^\circ$, while slightly contaminated products melted at $73-74^\circ$, as reported by Nagel.^{9,10} **Method II.**—0.2 g. of the pure isocyanate was suspended in 100 ml. of water, and refluxed at 100° for three hours. The solution was then allowed to cool to room temperature, filtered and the crude urethan thus obtained was purified as above. Fractional crystallization from ethyl acetate yielded a product melting at $78-79^\circ$, which showed no lowering in melting point when mixed with different proportions of the urethan prepared from the azide.

8,9,15-Trihydroxypentadecyl Amine.—A suspension of one gram of the pure isocyanate in 100 ml. of a 50% aqueous sodium hydroxide solution was refluxed for four hours on a hot plate. The solution was then cooled to room temperature and filtered through a sintered glass funnel. The residue was washed free of all adhering alkali with water, dried on an unglazed plate and fractionally crystallized from hot ethyl alcohol, yield 65%; m. p. $146-147^\circ$. *Anal.* Calcd. for $C_{18}H_{35}O_3N$: C, 65.45; H, 12.00; N, 5.09. Found: C, 65.39, 65.20; H, 11.66, 11.68; N, 5.29, 5.21. The purified product was completely soluble in hydrochloric acid and ethyl alcohol, but was insoluble in ether, petroleum ether, ethyl acetate and water.

Picrate of 8,9,15-Trihydroxypentadecyl Amine.—A solution of 1 g. of the purified amine dissolved in a 10% alcoholic solution of picric acid was evaporated to dryness on a water-bath. The solid product was extracted with hot benzene until a test portion of the extract showed the absence of even traces of the acid. The picrate was then thrice crystallized from ethyl alcohol and the purified product found to melt with decomposition at $118-119^\circ$. *Anal.* Calcd. for $C_{21}H_{40}O_{10}N_4$: C, 50.00; H, 7.14; N, 11.11. Found: C, 49.74, 49.83; H, 7.32, 7.39; N, 10.98, 10.89.

Summary

1. This investigation has demonstrated that it is possible to prepare a polyhydroxyamine from a polyhydroxycarboxylic acid by means of the Naegeli-Curtius series of reactions. The method has many possibilities of use in the further study of the chemical structure of shellac acids.

2. 8,9,15-Trihydroxypentadecyl isocyanate and 8,9,15-trihydroxypentadecylamine were prepared for the first time. These compounds are colorless solids which melt at $103.5-104.5^\circ$ and $146-147^\circ$, respectively.

3. The isocyanate reacts with water to produce N,N'-bis-8,9,15-trihydroxypentadecylurea, and with alcohol, 8,9,15-trihydroxypentadecylurethan. Both of these compounds were identical with those obtained by Nagel who prepared them from the azide. The pure urethan has a melting point of $78-79^\circ$ instead of $73-74^\circ$ as previously reported.

4. Aleurityl azide is relatively stable at room

temperature when obtained pure. It decomposes at 52° as compared with the crude product which explodes if heated rapidly to 50°. The pure azide

does not readily detonate when attacked with a hammer.

BROOKLYN, NEW YORK

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A Method for Standardization of Chromatographic Analysis¹

BY ARTHUR L. LEROSSEN

The chromatographic method of Tswett has been applied to many problems of the chemist with great success, making possible separations which could not be attained satisfactorily by any other means. As the use of this method increases it is desirable to obtain quantitative data for the comparison of adsorbents and the behavior of the adsorbed substances on these materials. A quantitative treatment of chromatography should be helpful in determining the best conditions for a given operation and in standardizing the properties of adsorbents.

Good books on chromatography are now available (Zechmeister,² Strain³). Theories of chromatography have been developed by Wilson⁴ and by Martin and Synge,⁵ and measurements of a quantitative nature have been made by Cassidy.^{6,7} Brockmann and Schodder⁸ have suggested a method of standardizing the adsorption affinity of certain alumina samples, using azo dye mixtures.

The investigations herein recorded were undertaken primarily with the purpose of ascertaining how far it is possible to determine the relative position of carotenoids on the adsorbent column by separate measurements of the rate of movement for each pigment.

It was first necessary to investigate the flow of the developing solvent through the adsorbent. The velocity of flow was found to vary directly with the pressure difference between the ends of

the column, inversely with the length of the column and to be essentially independent of the diameter of the tube (tubes of diameter 17, 43, and 70 mm. gave almost equal rates for columns of the same length). The exact nature of each of these dependencies has not been thoroughly studied.

The change of rate of flow with time was of more importance. Table I illustrates the behavior of calcium hydroxide columns. It is evident that the flow becomes constant after an initial decrease, a uniform rate being obtained soon after the solvent has reached the bottom of the column.

Three terms will now be introduced to simplify the following discussion: S = length of adsorbent column containing one unit volume of solvent/length of tube required to contain the same volume of solvent; V_c = rate of flow of developing solvent through the column when a state of constant flow has been reached (mm./min.); R = rate of movement of adsorbate zone (mm./min.)/rate of flow of developing solvent (V_c).

The ratio S may be of importance in characterizing the packing of the column, and, moreover, it gives the percentage of the tube volume occupied by the adsorbent (% volume adsorbent = $100(S-1)/S$). There is a variation in the degree of packing throughout the column; S was found to vary

TABLE I

RATE OF BENZENE FLOW THROUGH CALCIUM HYDROXIDE COLUMNS (17 MM. IN DIAMETER AND 150 ± 5 MM. LONG).

The data given indicate flow in mm. column length/min. Time was measured from the instant the solvent was poured on the column.^a

Column no.	Time, min.									
	1	5	10	15	20	25	30	35	40	
1	32.0	14.5	9.0	7.1	6.4	6.3	6.3	
2	43.0	15.5	9.8	7.1	7.0	7.0	7.0	
3	36.0	13.5	7.8	...	7.6	7.5	7.5	7.5	...	
4	42.0	15.3	7.6	7.7	7.7	7.7	7.7	
5	43.0	13.8	8.8	...	8.4	8.2	8.2	8.2	8.2	
6	42.0	16.0	13.0	8.1	8.1	8.1	8.1	

^a The solvent reached the bottom of the column in about twelve minutes.

(1) Presented before the Division of Analytical and Micro Chemistry of the American Chemical Society at the Memphis meeting, April, 1942.

(2) L. Zechmeister and L. Chohnoky, "Principles and Practice of Chromatography," John Wiley and Sons, Inc., New York, N. Y., 1941.

(3) H. H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1941.

(4) J. N. Wilson, *THIS JOURNAL*, **62**, 1583 (1940).

(5) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

(6) H. G. Cassidy and S. E. Wood, *THIS JOURNAL*, **63**, 2628 (1941).

(7) H. G. Cassidy, *ibid.*, **63**, 2735 (1941).

(8) H. Brockmann and H. Schodder, *Ber.*, **74**, 73 (1941).