acetic acid; m. p. 367° (cor.). This acid is insoluble in water and in the common organic solvents; attempts to form a picrate failed.

Anal. Calcd. for $C_{20}H_{12}N_2O_4$: neut. equiv., 172.16; C, 69.76; H, 3.51; N, 8.14. Found: neut. equiv., 174.4; C, 69.64; H, 3.61; N, 8.14.

2-Phenyl-3-ethylcinchoninic Acid (XIII).[14] In a similar manner, from 40 g. (0.27 mole) of isatin, 43 g. (0.27 mole) of phenyl *n*-propyl ketone, and 200 g. of 33% potassium hydroxide solution, was obtained 40 g. (55% yield) of crude acid. After recrystallization from acetone this compound melts at 286° (cor.); the picrate melts at 147° (cor.).

Anal. Calcd. for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.41; H, 5.59; N, 5.04.

Summary

1. The Pfitzinger reaction has been extended to include the production of two cinchoninic acid derivatives containing an alkoxyalkyl substituent at the 2-position through utilization of alkoxyalkyl ketones of type $CH_3OCH(CH_3)COR$.

2. These acids suffer cleavage of their ether linkage by action of concentrated hydriodic acid and red phosphorus, yet resist reduction.

3. The acids are decarboxylated by heating above their melting points, and, by action of tin with hydrochloric acid, undergo reduction of the pyridine nucleus and dealkylation of the ether group.

4. Bis-2-cinchoninic acid and 2-phenyl-3-ethylcinchoninic acid have been prepared and converted into their diethylamides.

5. Several substituted amides of these cinchoninic acids have been prepared and shown elsewhere not to possess antimalarial activity.

(14) Listed by von Oettingen, ref. 7, page 92.

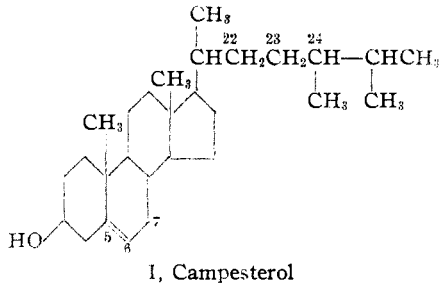
AUSTIN, TEXAS RECEIVED MAY 18, 1942

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

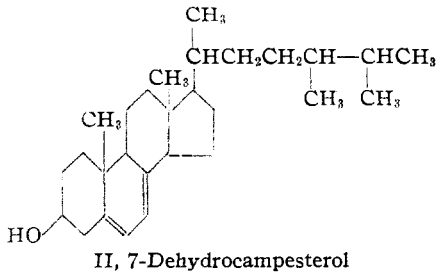
7-Dehydrocampesterol, a New Provitamin D

BY WILLIAM L. RUIGH

The relationship between the structure of the side chain of the D vitamins and their antirachitic activity has been the subject of a number of investigations. The isolation[1] of a new phytosterol, campesterol (I), and the determination of its structure[2] as the C-24 epimer of Δ^5-ergostenol, suggested a new approach to the problem.



I, Campesterol



II, 7-Dehydrocampesterol

(1) Fernholz and MacPhillamy, THIS JOURNAL, **63**, 1155 (1941).
(2) Fernholz and Ruigh, *ibid.*, **63**, 1157 (1941).

Campesteryl acetate was converted by the conventional method[3] via the 7-keto compound into 7(α)-benzoxycampesteryl benzoate. The usual method of preparing 7-dehydrosterols was modified at this point by selectively hydrolyzing the dibenzoate to the 7-monobenzoate and cleaving the latter into benzoic acid and free 7-dehydrocampesterol[4] II. Irradiation of this compound with ultraviolet light gave a resin, the antirachitic activity of which determined by the line test on rats was 725,000 international units per gram. The product obtained from ergosterol under identical conditions assayed at 7,000,000 I. U. per gram corresponding to a yield of 17.5% vitamin D_2 (40,000,000 I. U. per gram). Assuming the same extent of conversion in both cases the potency of the vitamin from 7-dehydrocampesterol is estimated to be 4,100,000 I. U. per gram, which is thus only 10% of the potency of vitamin D_2. Due to lack of sufficient material no attempt was made to isolate the new vitamin in crystalline form.

Vitamin D_4, prepared from 22,23-dihydroergosterol by Windaus and Trautmann,[5] was reported to have from 20,000,000 to 30,000,000

(3) Windaus, Lettré and Schenck, *Ann.*, **520**, 98 (1935).
(4) Wintersteiner and Ruigh, THIS JOURNAL, **64**, 1177 (1942).
(5) Windaus and Trautmann, *Z. physiol. Chem.*, **247**, 185 (1937).

international units per gram. On the basis of the comparative data available, the antirachitic substance from 7-dehydrocampesterol has only one-sixth the activity of vitamin D_4. The two vitamins are stereoisomers differing only in the configuration of carbon atom twenty-four in the side-chain. It is thus apparent that the difference in antirachitic activity due to stereoisomerism on C-24 is considerably greater than the comparatively slight difference (25–50%) caused by the presence or absence of the side chain double bond in vitamins D_2 and D_4, respectively.

The demonstration that 7-dehydrocampesterol can function as a provitamin is also of interest in connection with the claim advanced by Wunderlich[6] and later by Bills[7] that 7-dehydrositosterol can be activated by ultraviolet light. The starting material employed by Wunderlich for the preparation of the 7-dehydrositosterol was the sterol fraction from soy-bean which remained after the removal of the stigmasterol as the tetrabromide. From a similar fraction 3% of pure campesterol was later isolated in this Laboratory[1] and has served as the starting material for the present work. It seems reasonable to assume that Wunderlich's sitosterol contained 3% or more campesterol and possibly other as yet unisolated C-28 sterols such as 22,23-dihydrobrassicasterol.[8] It is, therefore, probable that a part of the antirachitic activity possessed by Wunderlich's product from sitosterol was due to the presence of vitamin derived from campesterol. In view of the fact that irradiated 7-dehydrostigmasterol is practically devoid of vitamin D activity,[9,10,11] the possibility will now have to be considered that the same may be true of pure 7-dehydrositosterol and of C-29 sterols in general. It is clear that this question cannot be unequivocally answered until Wunderlich's work has been repeated with a sitosterol entirely free from C-28 sterols.

The author wishes to thank Dr. O. Wintersteiner for his interest and encouragement during the course of this work, Dr. A. Black of the Squibb Vitamin Research Laboratory for the biological assays and the benefit of his experience on the irradiation of 7-dehydrosterols, Dr. N. H. Coy of the same laboratory for absorption spectra and assays and Mr. Karl Reinhardt for his efficient technical assistance. All microanalyses were performed by Mr. J. F. Alicino, Fordham University.

(6) Wunderlich, *J. physiol. Chem.*, **241**, 116 (1936).
(7) Bills, *J. Am. Med. Assoc.*, **108**, 13 (1937).
(8) Fernholz and Ruigh, THIS JOURNAL, **62**, 3346 (1940).
(9) Linsert, *Z. physiol. Chem.*, **241**, 125 (1936).
(10) Grab, *ibid.*, **243**, 63 (1936).
(11) Haslewood, *Biochem. J.*, **33**, 454 (1939).

Experimental

7-Ketocampesteryl Acetate.—Forty grams of campesterol, m. p. 156.5–158°, isolated from soy-bean oil[1] was converted to the acetate, m. p. 137–138°. The acetate was oxidized with chromic acid in glacial acetic acid following the method of Windaus, Lettré and Schenck[3] for the preparation of 7-ketocholesterol acetate. Fourteen grams of crude product, m. p. 168–80°, was obtained which on repeated recrystallization from alcohol yielded 7.4 g. of pure 7-ketocampesteryl acetate, needles, m. p. 177–178°; $[\alpha]^{24}D$ −88.6° (1.18% in chloroform).

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59. Found: C, 78.72, 78.80; H, 10.47, 10.60.

7(α)-Benzoxycampesteryl Benzoate.—The reduction with aluminum isopropylate and isopropyl alcohol of 7 g. of 7-ketocampesteryl acetate yielded 4.7 g. of crude hexane-precipitable diol. Benzoylation in pyridine gave 3.8 g. of crude dibenzoate, m. p. 171–172°, from which was obtained pure benzoxycampesteryl benzoate by repeated recrystallization from acetone, needles, m. p. 176.5–177.5°; $[\alpha]^{23}D$ +96.6° (0.81% in chloroform).

Anal. Calcd. for $C_{42}H_{56}O_4$: C, 80.72; H, 9.03. Found: C, 80.90; H, 9.38.

7(α)-Benzoxycampesterol.—To 2 g. of the dibenzoate dissolved in 40 cc. of benzene a solution of 1.33 g. of sodium methylate in 66 cc. of dry methanol was added. The mixture was allowed to stand at room temperature for sixteen hours and worked up as described before[4] for the corresponding cholesterol derivative. The chromatographed product, 1.60 g., crystallized from benzene–hexane in filamentous needles. 7(α)-Benzoxycampesterol melts at 143–145° after sintering to a glassy solid at 126–130°; $[\alpha]^{26}D$ +115.0° (1.16% in chloroform). The analytical sample on drying for two hours at 108° in a high vacuum showed a loss in weight of 2.07%.

Anal. Calcd. for $C_{35}H_{52}O_3$: C, 80.72; H, 10.07. Found: C, 80.40, 80.38; H, 9.87, 9.94.

7-Dehydrocampesteryl Benzoate.—One gram of 7(α)-benzoxycampesterol was refluxed with dimethylaniline and worked up as previously described.[4] The digitonide on decomposition by pyridine–ether yielded 564 mg. of impure 7-dehydrocampesterol; leaflets from acetone, m. p. 148–149.5°[12] $[\alpha]^{23}D$ −91.0°, ϵ_{282} = 9350, corresponding to 81% of the absorption at 282 mμ given by ergosterol. The crude dehydrocampesterol (475 mg.) was benzoylated in pyridine and after seven recrystallizations from benzene–alcohol 229 mg. of 7-dehydrocampesteryl benzoate was obtained in the form of fine needles. The compound melted at 156–157° to a cloudy liquid which cleared sharply at 164°. This degree of purity was actually attained on the fourth crystallization and three further crystallizations did not change the melting point behavior. The compound gave positive Tortelli–Jaffé and Rosenheim trichloroacetic acid reactions, $[\alpha]^{23}D$ −50.2 (1.0% in chloroform).

(12) All melting points of dehydro derivatives were taken in sealed evacuated tubes after drying for half an hour under a high vacuum at 107°.

Anal. Calcd. for $C_{35}H_{50}O_2$: 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°, $[\alpha]^{25}D$ −109.0° (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 mμ and 282 mμ 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 D_2 rather than vitamin D_3 in that it is relatively less active when assayed by the chick test.

NEW BRUNSWICK, N. J. RECEIVED JUNE 6, 1942

[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 Reactions[1]

BY ARTHUR L. DAVIS[2] 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 method[6] 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] Naegeli[7] 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 $C_{15}H_{31}O_3$ 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, L. Grüntuch and P. Lendorff, *Helv. Chim. Acta* **12**, 227 (1929).