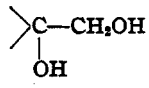
[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Cafesterol. II[1]

BY P. N. CHAKRAVORTY, MILDRED M. WESNER AND ROBERT H. LEVIN[2]

From the unsaponifiable fraction of green coffee oil, Slotta and Neisser[3] isolated a substance, $C_{20}H_{28}O_3$, which is quite sensitive to light, air and traces of acid. On the basis of certain color reactions and a positive test for estrogenic activity, these authors suggested that the compound was a steroid and named it cafesterol.[4] Of the three oxygen atoms in the molecule, two were fixed in a glycolic group



by Slotta and Neisser[3] and also by Hauptmann and França,[5] who showed that one carbon of the glycol is part of a ring. The third oxygen, being unreactive, was postulated as being in position 11 of the sterol nucleus, by analogy with corticosterone. It was the possibility of obtaining substances of the cortical hormone series that turned our attention to cafesterol.

After our work was well under way, there appeared several papers on this subject by a Swiss group,[6,7] and, more recently, another paper by Hauptmann and França.[8] We have independently corroborated some of that work and wish to record some additional observations.

In agreement with Wettstein, Fritzsche, Hunziker and Miescher,[6] it was found that cafesterol acetate on hydrogenation in the presence of palladium on charcoal readily takes up two moles of hydrogen to form tetrahydrocafesterol acetate (or ox-cafestandiol monoacetate),[6] m. p. 152–153°. When cafesterol was similarly treated, the resulting tetrahydrocafesterol was difficult to purify, although it crystallized well from various solvents. It is possible that some of Hauptmann and França's isomer of tetrahydrocafesterol, m. p. 188°, was formed. Acetylation of our product gave tetrahydrocafesterol acetate, m. p. 152–153°. Tetrahydrocafesterol, m. p. 123–143°, and crude cafesterol, m. p. 147–151°, each in 2 mg. doses, were positive in the Kahnt and Doisy test for estrogenic activity.[9]

Cafesterol reacts very readily with maleic anhydride when warmed in benzene at 35–40° for thirty minutes. Boiling such a solution promptly causes decomposition. On standing overnight at room temperature cafesterol–maleic anhydride, m. p. 190–192°, is formed quantitatively. The fact that cafesterol forms a maleic anhydride addition compound under such mild conditions indicates that it has an actual conjugated double bond system, since it is very unlikely that the formation of the adduct is preceded by rearrangement (*i. e.*, like that occurring when the diterpene, abietic acid, undergoes a Diels–Alder reaction[10]).

Cafesterol exhibits an absorption maximum at *ca.* 290 mμ[6,8] further establishing the presence of a conjugated double bond system.[11] The Swiss group[6] report the extinction coefficient at 290 mμ to be 560 and state that less pure preparations of cafesterol exhibit greater extinction coefficients. In connection with a spectrographic study of the ultraviolet irradiation of cafesterol acetate, m. p. 162–165°, we found that E_{290} = 3500 initially. In view of the results of Wettstein and co-workers,[6] we repeated our experiment using cafesterol acetate which was sublimed at 0.0008 mm. and 165–175°, recrystallized, resublimed at 0.001 mm. and 125–130°, and taken directly for spectrographic study. The results of this experiment are given in Fig. 1. This sample of cafesterol acetate exhibited exactly the same type of curve as that described by Wettstein and co-workers[6] and by Hauptmann and França.[8] However, its E_{290} value is 6300 or almost double that of our previous sample of cafesterol acetate,

(1) Presented in part before the Organic Division of the American Chemical Society, Buffalo, N. Y., September, 1942. Paper I, Chakravorty and Wesner, THIS JOURNAL, **64**, 2235 (1942).

(2) The preparation of this study for publication was carried out by R. H. L. and M. M. W. after the untimely death of Dr. Chakravorty.

(3) Slotta and Neisser, *Ber.*, **71**, 1991, 2342 (1938).

(4) Previously the unsaponifiable fraction of the coffee bean had been investigated by von Noel and Dannmeyer, *Strahlentherapie*, **32**, 769 (1929); *ibid.*, **38**, 583 (1930); and Bengis and Anderson, *J. Biol. Chem.*, **97**, 99 (1932). However, neither of these groups succeeded in obtaining a completely pure product, and did little toward its characterization.

(5) Hauptmann and França, *Z. physiol. Chem.*, **259**, 245 (1939).

(6) Wettstein, Fritzsche, Hunziker and Miescher, *Helv. Chim. Acta*, **24**, 332E (1941).

(7) Wettstein and Miescher, *ibid.*, **25**, 718 (1942).

(8) Hauptmann and França, THIS JOURNAL, **65**, 81 (1943).

(9) Since Wettstein and co-workers,[6] and also Hauptmann and França[8] found cafesterol to be inactive estrogenically at higher doses, it is probable that the low order of activity reported here, and by Slotta and Neisser,[3] is due to a minute trace of impurity.

(10) Ruzicka and Bacon, *Chemistry & Industry*, **55**, 546 (1936).

(11) Dimroth, *Angew. Chem.*, **52**, 545 (1939).

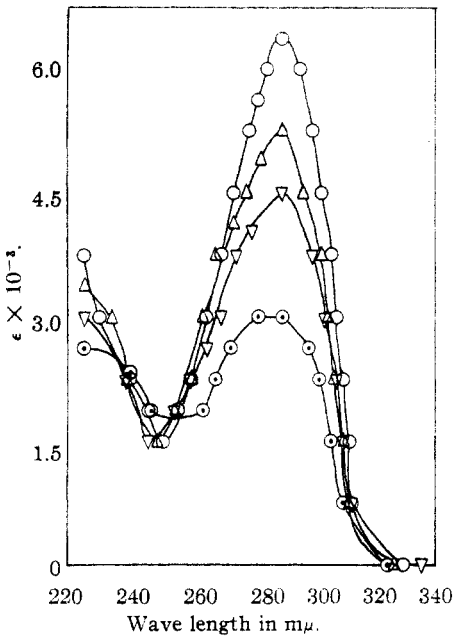
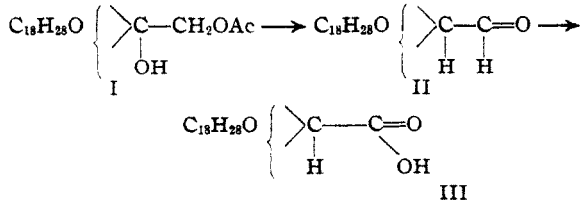


Fig. 1.—Absorption spectra of cafesterol acetate (ox-cafestadiendiol monoacetate) at successive stages of irradiation: O, after 0 min.; △, after 1.5 min.; ▽, after 4.5 min.; ⊙, after 10.0 min.

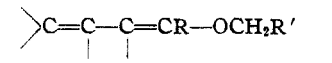
and more than ten times the intensity of Wettstein's cafesterol. It will be observed that irradiation[12] caused a gradual decline in the E_{290} value, indicating disappearance of the conjugated double bond system.[11] There was also a lowering of the value of E at 225 mμ.

Wettstein and co-workers[6] confirmed the statement of previous investigators[3,5] that zinc dust distillation of cafesterol acetate gives an aldehyde, but in very poor yield. Using tetrahydrocafesterol acetate (ox-cafestandiolmonoacetate) (I) we have found the proper conditions for this decomposition and have succeeded in obtaining the aldehyde (II)



and the corresponding acid (III) in excellent yields. The aldehyde forms a semicarbazone, m. p. 217–218°, and a 2,4-dinitrophenylhydrazone, m. p. 231–233°. The acid, which we are calling ox-cafestanic acid, has a m. p. of 260–262°, and forms a methyl ester, m. p. 123.5–124.5°. The methyl ester was recovered unchanged after heating with acetic anhydride, indicating that no acylable hydroxyl group is present in the molecule.

Ox-cafestanic acid was recovered unchanged after treatment with platinum oxide and hydrogen at room temperature and at 60° with a few drops of hydrochloric acid added. According to Wettstein and Miescher[7] the inert oxygen in cafesterol is probably present as an enol ether. This indicates the existence of the grouping



in cafesterol and explains the relative ease of hydrogenolysis of the ether linkage.[13] Since the ox-cafestanic acid is completely saturated, it is to be expected that cleavage of the ether by hydrogen would be more difficult. Ox-cafestanic acid in 20-mg. doses was inactive in the Ingle work performance test for cortin activity[14] and contained less than one rat unit per 5 mg. when tested by the Kahnt and Doisy method for estrogenic activity.

On the basis of all work to date we are inclined to agree with Wettstein and Miescher[7] and others that cafesterol is not a steroid and is probably diterpenoid in nature. The sodium and alcohol treatment of cafesterol[1] will be discussed fully in a forthcoming publication.

Acknowledgments.—We wish to acknowledge the assistance of Dr. G. Reed of our Nutrition Department who made the spectrographic studies and kindly helped us to interpret the data. We are indebted to J. W. Nelson and Dr. G. F. Cartland of our Endocrinology Department for the estrogen assays, and to Dr. D. J. Ingle of the same department for the cortin assay.

Experimental[15]

Isolation of Cafesterol.—The method of Slotta and Neisser[8] was modified as follows: coffee oil (130 g.), obtained from green Santos Bourbon beans by chloroform extraction,[16] was dissolved in ether and the solution filtered to remove an insoluble substance which otherwise interfered with the isolation of cafesterol in the final step. The ether was removed by distillation, and the oil dissolved in

(12) Irradiation by a mercury arc lamp. Cafestrol dissolved in isopropyl alcohol and air excluded.

(13) Adkins, "Reactions of Hydrogen with Organic Compounds over Copper-Chromium Oxide and Nickel Catalysts," The University of Wisconsin Press, Madison, 1937, p. 73.

(14) Ingle, *Endocrinology*, **26**, 472 (1940).

(15) Microanalyses by H. Emerson of The Upjohn Company and J. F. Alicino of Fordham University and The Squibb Institute.

(16) The green beans in lots of 25 to 50 lb. were ground and extracted in the milling and percolating departments, and the oil kept in the refrigerator to be used as needed.

600 cc. of acetone. After the addition of 4.5 g. of Super-Cel it was saponified with 110 g. of finely ground barium hydroxide in 700 cc. of carbon dioxide-free water. The mixture was allowed to reflux on the steam-bath overnight.

The barium salt was separated from the hot solution by filtration, ground fine in acetone, and digested two or three times in *ca.* 300 cc. of this solvent. All the filtrates were combined and the acetone separated from the mixture by distillation. To ensure complete saponification of the oil, a few cubic centimeters of 2 *N* sodium hydroxide and 500 cc. of alcohol were added and the mixture was refluxed for another hour. The alcohol was removed *in vacuo* and the cooled, aqueous solution neutralized with a stream of carbon dioxide.

The precipitate, consisting of carbonate, cafesterol and impurities, was collected on a filter, and the filtrate extracted with two 150-cc. portions of ether. The aqueous fraction was discarded. The solid material was digested with two 400-cc. portions of acetone and the acetone concentrated to a very small volume, cooled and poured into the ether extract which had been diluted to *ca.* 1200 cc. The ether was washed once with water, dried with anhydrous sodium sulfate and treated with Darco (acid free). The filtered ether solution was evaporated to dryness and the last trace of solvent removed *in vacuo*.

The residue, a viscous, yellow oil, was digested with several 300–400 cc. portions of hexane (Skelly-Solve B) until the solution became definitely cloudy. The liquid was decanted, and the cafesterol allowed to crystallize. As soon as the residue had become solid, the extraction was continued in a Soxhlet until complete. The total yield was 5.4 g. of cafesterol, m. p. 147–151°, $[\alpha]^{30}D$ in chloroform −156°.[17] This compound gives the characteristic green to blue color reaction described by Slotta and Neisser.[3] Several recrystallizations from hexane gave cafesterol, m. p. 158–160°.

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.90; H, 8.77.

In a series of more than twenty runs this procedure gave yields of 4–7 g. or 0.4–0.6% of the green coffee.

Cafesterol Acetate.—Acetic anhydride (16 cc.) was added to 3.22 g. (0.010 mole) of cafesterol in 40 cc. of dry pyridine. The yellow solution was warmed gently on the steam-bath for about forty-five minutes and worked up in the usual manner. The yield of crude acetate was 2.04 g. (56%). Several recrystallizations from methanol gave a compound which melted 160–165°.

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.43. Found: C, 73.78; H, 8.63.

Attempts were made to prepare a benzoate by the usual methods, but no crystalline product could be obtained.

Hydrogenation of Cafesterol Acetate with Palladium on Charcoal.—A solution of 1.7 g. (0.0047 mole) of cafesterol acetate (m. p. 150–154°) in 125 cc. of absolute alcohol was shaken with *ca.* 0.5 g. of palladized Norite[18] (acid free) in an Adams hydrogenator at 34 lb. and room temperature for two hours. The product weighed 1.0 g. (59%). After several crystallizations from dilute acetone and dilute methanol it was dried in an Abderhalden pistol at 117°, m. p. 152–154.5°; $[\alpha]^{29}D$ in chloroform −20.4°; mixed melting points with cafesterol and with cafesterol acetate showed depressions. This compound gives a negative color reaction with concentrated hydrochloric acid.[3]

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.46. Found: C, 72.80; H, 9.18.

Tetrahydrocafesterol was prepared by saponification of the acetate for thirty minutes in an aqueous-alcoholic potassium hydroxide solution. Crystallization as above gave a compound, m. p. 154.5–157°, which formed no precipitate with digitonin.

Anal. Calcd. for $C_{20}H_{32}O_3$: C, 74.96; H, 10 07. Found: C, 75.03; H, 9.83.

Reaction of Cafesterol with Maleic Anhydride.—A solution of 3.66 g. (0.0012 mole) of crude cafesterol, m. p. 146–151°, in 80 cc. of thiophene-free benzene was allowed to react at 35–40° with 2.5 g. (0.0026 mole) of maleic anhydride similarly dissolved. In ten minutes the solution had become cloudy and in thirty minutes an appreciable amount of precipitate had formed. After standing overnight at room temperature the precipitate of cafesterol maleic anhydride adduct was separated, washed with benzene and dried on the steam-bath; yield, 4.5 g. (94% of the theoretical). Several crystallizations from acetone yielded a product of m. p. 190–192°; $[\alpha]^{29}D$ in acetone −43°.

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.52; H, 7.06.

Ox-cafestanic Acid.—An intimate mixture of tetrahydrocafesterol acetate (0.82 g., 0.0023 mole) (m. p. >145°) and zinc dust (13.1 g.) was placed in a retort of about 25-cc. capacity, and heated in an oil-bath at 180–200° and 1.5 mm. for twenty minutes. The reaction product was distilled by reducing the pressure to 0.02 mm. and heating cautiously with a free flame. A colorless, amorphous solid collected in the arm of the retort, and was washed out with ether. After filtering to remove zinc dust, the colorless solution was taken to dryness. The aldehyde (0.59 g., 85%) was not purified, but was dissolved in acetone previously distilled over potassium permanganate, and the solution heated just to boiling. Potassium permanganate, dissolved in similarly purified acetone, was added in slight excess and the reaction mixture left at room temperature overnight.

The manganese dioxide was removed by filtration, and most of the acetone boiled off. A few cubic centimeters of dilute sodium hydroxide and about 10 cc. of alcohol were added to the hot solution which was then cooled and filtered through a sintered glass funnel. Concentration and acidification with hydrochloric acid produced a flocculent precipitate which became almost crystalline on warming, wt. 0.46 g. (74% of the theoretical). The over-all yield of ox-cafestanic acid from tetrahydrocafesterol acetate is 63%. The acid was recrystallized several times from acetone and melted 251–253°.

A very pure sample was obtained by redissolving in dilute potassium hydroxide solution, filtering, and acidifying. Repeated crystallization from acetone gave crystals melting at 260–262°; $[\alpha]^{30}D$ in chloroform −39.7°.

(17) After standing two weeks, the chloroform solution used for rotation had become dark yellow, indicating some decomposition. The reading was repeated: $[\alpha]^{31}D$ −161°.

(18) N. Levin, Thesis, Doctor of Philosophy, University of Maryland, 1941. Prepared by using 1 g. of Norite, 100 cc. of 0.5 *N* aqueous sodium acetate and 0.2 g. of palladium chloride.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.33; H, 9.54.

The **methyl ester** was prepared with diazomethane, and recrystallized from dilute acetone, m. p. 123.5–124.5°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.98; H, 9.79.

In order to make derivatives of the aldehyde, 1.3 g. of tetrahydrocafesterol acetate was distilled with zinc dust, the reaction product taken up in alcohol, and divided into two parts.

The **semicarbazone** was prepared according to the method of Shriner and Fuson.[19] Successive crystallizations from dilute methanol, dilute ethanol and finally methanol–benzene gave a compound melting at 217–218°.

Anal. Calcd. for $C_{21}H_{33}O_2N_3$: C, 70.16; H, 9.25; N, 11.69. Found: C, 70.22; H, 9.50; N, 11.75.

The ***p*-nitrophenylhydrazone,** prepared according to the method of Shriner and Fuson[19] was recrystallized from dilute alcohol, and ethyl acetate–methanol melting finally at 231–233°.

(19) Shriner and Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 2d ed., 1940.

Anal. Calcd. for $C_{28}H_{35}O_3N_3$: N, 9.60. Found: N, 9.78.

Preliminary experiments showed that if the reaction with zinc dust was allowed to take place at pressures below 1.0 mm., or if the heating for distillation was too prolonged, the yield of aldehyde decreased considerably.

Ox-cafestanic acid was recovered unchanged when treated with hydrogen in acetic acid in the presence of platinum oxide catalyst and concentrated hydrochloric acid for five hours at 50 lb. and 60°.

Summary

Cafesterol contains a conjugated double-bond system as indicated by the addition of two moles of hydrogen, the facile formation of a maleic anhydride addition compound, and absorption spectra data.

The glycolic group in tetrahydrocafesterol acetate has been converted, by zinc dust distillation, into the aldehyde, ox-cafestanal; and subsequently, by oxidation, to ox-cafestanic acid.

KALAMAZOO, MICHIGAN RECEIVED FEBRUARY 15, 1943

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF COLUMBIA UNIVERSITY]

Amidino Arsenicals. I. *p*-Amidinophenylarsonic Acid and 4,4'-Diamidinoarsenobenzene

BY FRED LINSKER[1] AND MARSTON TAYLOR BOGERT

Organic arsenicals, such as the arsphenamines (salvarsans), tryparsamide, acetarsone, mapharsen and carbarsone, have proved valuable in the treatment of syphilis, trypanosomiasis and amebiasis. Some also possess antimalarial properties. In the 1942 issue of the authoritative New and Nonofficial Remedies, of the American Medical Association, it is stated (p. 169) that "The diseases in which arsenic therapy has proved useful are particularly those caused by protozoa. Inorganic arsenic will kill protozoa, but it cannot be administered so as to reach the protozoa in fatal quantity. In the body, the organic compounds are less toxic to mammals and more toxic to protozoan parasites. In this way they become available for combating trypanosomiasis, treponematosis, spirillosis and other protozoan infections."

As the result of the investigations of King, Yorke, Lourie, Hughes, Ewins and many others, as well as the May and Baker patents, a number of amidino derivatives have been found to possess useful curative properties for several tropical diseases, and a few are active against malaria.

(1) Merck Research Fellow at Columbia University, New York.

The synthesis of compounds carrying both the amidino group and arsenic in their molecules, would seem to offer good possibilities of discovering within such a class some products of therapeutic value in dealing with tropical protozoal diseases. We have therefore initiated investigations in this direction because, so far as we are aware, no amidino arsenicals have as yet been described in the literature, and the present paper reports some of our experiments in this field.

As will be noted from the Flow Sheet, these first experiments follow familiar lines, paralleling syntheses and structures of well-known medicinals, but in which an amino has been replaced by an amidino group, an ethylene (—CH=CH—) by an arseno (—As=As—) union, or similar changes brought about, to yield organic amidino arsenicals as the final products.

One objection to arsphenamine (salvarsan), for example, has been always its acid reaction and consequent irritant effect when injected intravenously. It is so weak a base that its hydrochloride is easily hydrolyzed with liberation of hydrochloric acid. To protect the patient from this,