[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

Cafesterol. III.[1] Isocafesterol

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

Cafesterol is a polycyclic substance (probably terpenoid)[3] which is obtained from the non-saponifiable fraction of coffee oil.[4] It has the formula $C_{20}H_{28}O_3$ in which two of the oxygen atoms are fixed in a glycolic group.[4,5] The third oxygen is probably present as a cyclic enol ether[3] included in the grouping[1]

$$>C{=}C{-}C{=}CR{-}OCH_2R'$$

That cafesterol contains a highly reactive double bond system has been shown by its absorption spectrum curve, and by the facile formation of an addition compound with maleic anhydride.[1,6] However, both Slotta and Neisser,[4] and Wettstein and associates[6] have reported that the expected reduction of cafesterol by sodium and alcohol does not take place.

In this Laboratory, a more careful study of the treatment of cafesterol with a large excess of sodium and alcohol has revealed that cafesterol is isomerized to a new compound, without any reduction occurring.

The evidence for the formation of the new substance, which we have named isocafesterol, is as follows. Cafesterol is quite sensitive to light, air and traces of acid. If not kept in the dark in a desiccator it will turn yellow in less than a day. In alcoholic solution it gives a characteristic blue-green color with concentrated hydrochloric acid.[4] In contrast, isocafesterol is stable to light and air, and gives a pink color with hydrochloric acid. Yet, after repeated crystallizations from various solvents, it gives a m. p. of 156–159° and shows no mixed m. p. depression with cafesterol, m. p. 153–155°, and its acetate, m. p. 163–167°, shows no mixed m. p. depression with cafesteryl acetate, m. p. 162–165°.

Conclusive proof for the formation of a new compound is furnished by absorption spectrum studies (Fig. 1). Whereas cafesterol and its derivatives give curves with a maximum at 292 mμ,[1,6,7] isocafesterol exhibits no absorption in this region of the spectrum. At first it was supposed that a dihydrocafesterol had been formed by the sodium and alcohol treatment. To test this possibility, a variety of double bond determinations were carried out. With monoperphthalic acid, cafesterol took up two atoms of oxygen and isocafesterol absorbed two and a half atoms. Using bromine, both substances reacted with four moles of the reagent. Finally, quantitative hydrogenation with palladized charcoal catalyst resulted in the absorption of two moles of hydrogen with the formation of the same tetrahydrocafesterol (oxcafestandiol) obtained by the similar treatment of cafesterol.[1] With establishment of the fact that isocafesterol is not a reduction product of cafesterol, it was thought possible that the strongly alkaline solution formed by the reaction of sodium with alcohol had induced rearrangement of the double bonds to a non-

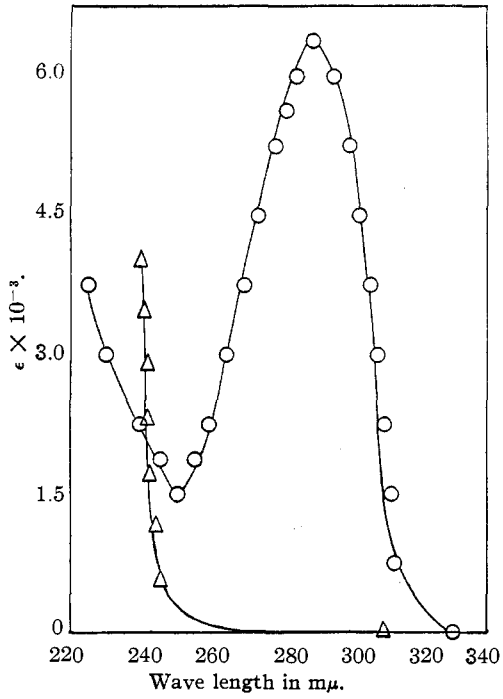


Fig. 1.—○, Absorption spectrum of cafesterol in isopropyl alcohol; △, absorption spectrum of isocafesterol in isopropyl alcohol.

(1) Paper II, Chakravorty, Wesner and Levin, THIS JOURNAL, **65**, 929 (1943).

(2) The development of this study and its preparation for publication was carried out under the direction of R. H. L. after the untimely death of Dr. Chakravorty.

(3) Wettstein and Miescher, *Helv. Chim. Acta*, **25**, 718 (1942).

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

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

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

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

conjugated system. Accordingly, cafesterol was treated with alcoholic sodium methoxide under the identical conditions used in the preparation of isocafesterol. The product gave an absorption curve indistinguishable from that of cafesterol, with a maximum at 292 mμ.

A Diels–Alder reaction using maleic anhydride and spectographically pure isocafesterol[8] revealed that the isomerization product reacts quantitatively with maleic anhydride at room temperature to give an adduct melting at 177–180°. A mixed m. p. with cafesterol–maleic anhydride, m. p. 185–190°, showed no depression. An attempt to acetylate isocafesterol–maleic anhydride with acetic anhydride in pyridine resulted in the decomposition of the adduct and the isolation of isocafesteryl acetate. Since Wettstein, *et al.*,[6] had reported that the cafesterol addition compound can be decomposed with the regeneration of cafesterol, this experiment indicated that cafesterol and isocafesterol probably do not form the same maleic anhydride adduct by isomerization of the double bond system. Proof of the non-identity of the adducts was established by a series of parallel experiments. Cafesterol–maleic anhydride was pyrolyzed at 150° and 0.01 mm. The sublimate gave a blue color with concentrated hydrochloric acid[4] and an absorption maximum at 292 mμ. A sample of pure isocafesterol was allowed to react with maleic anhydride. The adduct was pyrolyzed as above, giving isocafesterol, as indicated by a pink color with hydrochloric acid, absence of absorption in the region of 250–300 mμ, and analytical data.

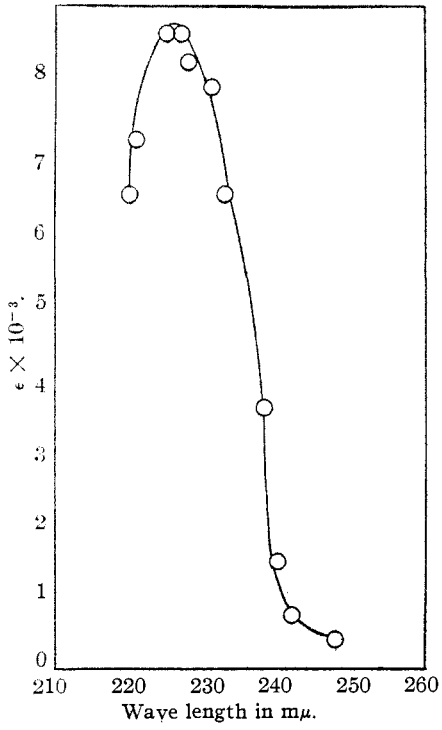


Fig. 2.—Absorption spectrum of isocafesterol in isopropyl alcohol.

Since isocafesterol takes part in the diene synthesis under such mild conditions, it is very likely that it possesses a conjugated double bond system. A careful examination of its absorption spectrum in the region of 220–250 mμ revealed a maximum at 226 mμ (Fig. 2). In comparison with data for other doubly unsaturated compounds[9] this observation indicates that the conjugated double bonds in isocafesterol are distributed between two rings or between a ring and a side chain. With an absorption maximum at 292 mμ, it is probable that the double bonds of cafesterol are present in a conjugated system contained in a single ring. The data summarized in Table I show unequivocally that cafesterol and isocafesterol are different

TABLE I

COMPARISON OF PHYSICAL AND CHEMICAL PROPERTIES

	Cafesterol	Isocafesterol
Free Compound		
Melting point, °C.	158–160[a]	156–159[a]
$[\alpha]^{30}D$	−107 ($CHCl_3$)	−108 ($CHCl_3$)
		−114 (acetone)
Color reaction with HCl[4]	Blue	Pink
Stability to air and light	Very unstable	Stable
Moles perphthalic acid / Moles of compound	2.1 2.0	2.6 2.3
Moles of bromine / Moles of compound	3.9	4.0 4.3
Moles of hydrogen / Moles of compound	2[b]	2[b]
Acetates, m. p.	160–165[c]	163–167[c]
Absorption maximum	292 mμ	226 mμ
Maleic Anhydride Addition Compound		
Reaction conditions	Mild	Mild
M. p., °C.	190–192	177–180
$[\alpha]^{30}D$	−43 (acetone)	−45 (acetone)
Adduct Decomposition Products		
Color reaction with HCl[4]	Blue	Pink
Absorption maximum	292 mμ	226 mμ

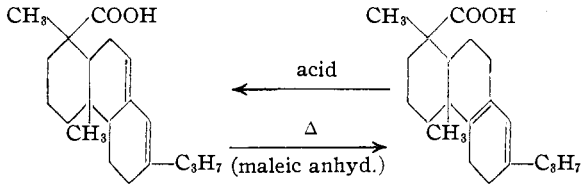
[a] No mixed m. p. depression. [b] Same tetrahydrocafesterol formed in each case. [c] No mixed m. p. depression.

(8) Chakravorty and Wesner, THIS JOURNAL, **64**, 2235 (1942).

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

compounds, although they exhibit a number of similar properties.

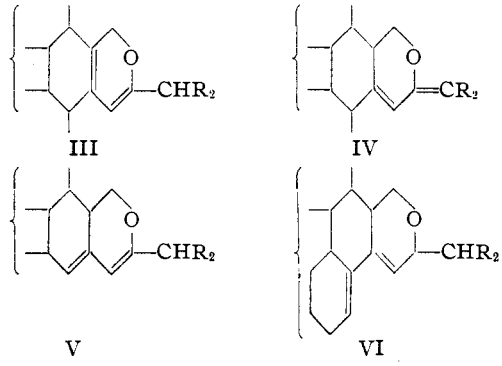
The isomerization of cafesterol to isocafesterol can be discussed in relation to the somewhat analogous interconversion of the diterpenes, abietic acid (I) and levopimaric acid (II).[10] Abietic



I II

acid shows an absorption at 237.5 mμ, is not reduced by sodium and alcohol, and does not add maleic anhydride until it has been isomerized by heat to levopimaric acid. The latter compound (II) is labile and readily converted into abietic acid (I) by the action of dilute acids. Levopimaric acid has an absorption maximum at 272.5 mμ, is reduced by sodium and alcohol, and readily adds maleic anhydride.[11]

In the case of cafesterol–isocafesterol, the isomerization takes place in a strongly basic reducing medium. Since isocafesterol does not give a blue color with concentrated hydrochloric acid, the isomerization is obviously not reversed by acids. Formula III is a possible representation of a partial structure for cafesterol. This fragment meets the requirements of all available evidence. It contains a cyclic enol ether attached to a methylene group and a conjugated double bond system, respectively. Formulas IV, V, and VI are possible rearrangement products



III IV

V VI

of III which would satisfy the spectrographic evidence.[9] However, V would not react with maleic anhydride, and VI would react only slowly. Little is known about polynuclear substances containing the fragment IV.[12] Such a formula (IV) would probably best explain the properties of isocafesterol. It should be emphasized that we have made no attempt in our speculations to fit together the diene-ether portion of the cafesterol molecule with that part containing the glycolic group attached to a five-membered ring.[6] Dehydrogenation studies on the more stable isocafesterol should offer a clue as to the basic carbon skeleton of the cafesterol molecule.

Because of other work, it is necessary for us to discontinue our studies on cafesterol at this time.

Experimental[13]

Preparation of Isocafesterol.—A solution of 3.0 g. (0.0095 mole) of cafesterol in 250 cc. of absolute alcohol was placed in a 1-liter, three-necked, round-bottom flask, fitted with a stirrer, reflux condenser, and calcium chloride tube. Twenty-seven grams of sodium (1.17 moles) was dropped into the refluxing solution over a period of three hours. Toward the end of the reaction an extra 100 cc. of absolute alcohol was added and the heating continued for another hour.

The reaction product was precipitated by pouring the mixture into 1300 cc. of ice water. The aqueous solution was extracted with 1 liter of ether in three portions. Most of the yellow coloring matter and all of the precipitate went into the ether layer, which was washed with sodium carbonate and then with water. Acidification of the aqueous fraction gave no precipitate. The ether solution was dried with anhydrous sodium sulfate, decolorized with Darco, and allowed to stand overnight. The colorless solution was filtered, concentrated to 50 cc., and diluted with water. The precipitate, collected on a filter and washed with dilute alcohol, melted at 147–157°; mixed m. p. with cafesterol, no depression; yield 2.38 g. or 79% of the theoretical.

In carrying out the color reaction, two to three drops of concentrated hydrochloric acid were added to a few crystals of the isocafesterol dissolved in alcohol.[4] The solution became yellow, changing gradually to deep pink when warmed. Further heating produced an orange tinge and a fine white precipitate.

In a few instances the crude sodium treated product gave a blue color test similar to that of cafesterol,[4] and also showed a slight absorption in the region of 250–300 mμ. However, after one crystallization from isopropyl alcohol or ethylene dichloride–hexane, the color test was pink, and the substance no longer showed any absorption above 250 mμ.

For purification, 2 g. of isocafesterol was boiled for several minutes in 50 cc. of hexane, and the undissolved solid was separated from the hot solution by filtration. The insoluble material melted at 156–159° and gave the

(10) Fieser and Campbell, THIS JOURNAL, **60**, 159 (1938), summarize some of the data on this subject. See also "Annual Reports of the Chemical Society (London)," Vol. XXXVIII, p. 187 (1941).

(11) The analogous 2,4-cholestadiene and cholesterylene-3,5 exhibit similar relationships to each other; Stavely and Bergmann, *J. Org. Chem.*, **1**, 567, 575 (1937).

(12) See Bann, Heilbron and Spring, *J. Chem. Soc.*, 1274 (1936), for the properties of 7-methylene cholesterol.

(13) Microanalyses by H. Emerson.

characteristic pink color test. Further crystallization from a variety of solvents and purification *via* the acetate failed to raise the m. p., $[\alpha]^{30}D$ in chloroform, $-108°$; in acetone, $-114°$.

Anal. Calcd. for $C_{26}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.90; H, 8.77.

For spectrographic analysis a 25 mg. per cent. solution of isocafesterol in absolute alcohol was used.

Isocafesteryl acetate was prepared from 0.34 g. of isocafesterol in dry pyridine by warming gently with 2.4 cc. of acetic anhydride for one hour. The reaction mixture was worked up in the usual manner, giving a product which melted at 156–163°. Four crystallizations from dilute acetone gave a substance of m. p. 163–167°. Drying in an Abderhalden pistol at 110° for several hours resulted in no change in the m. p. A mixed m. p. with cafesteryl acetate showed no depression.

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 73.71; H, 8.43. Found: C, 73.80; H, 8.14.

In double bond determinations using **monoperphthalic acid** in ether (seventy hrs., 5°), the amount of reagent absorbed per mole of substance was: 2.6, 2.3 moles for isocafesterol; 2.0, 2.1 moles for cafesterol; and 2.8 moles for ergosterol. **Bromine titrations** gave the following reaction per mole of substance: isocafesterol, 4.0, 4.3 moles; cafesterol, 3.9 moles; cholesterol, 1.1 moles.

Treatment of Cafesterol with Sodium Methoxide.—Cafesterol (0.30 g.) was refluxed with 10 g. of sodium methoxide following the experimental conditions of the sodium–alcohol treatment. Shiny yellow crystals were obtained which gave a strong absorption maximum at 292 mμ (27.2 mg. per cent. in absolute alcohol).

Hydrogenation of Isocafesterol with Palladium on Charcoal.—A solution of 1.61 g. (0.0051 mole) of isocafesterol in 125 cc. of absolute alcohol was shaken with palladized charcoal[14] in the presence of hydrogen for twelve hours at atmospheric pressure and room temperature. Two moles of hydrogen were absorbed, the first quickly, and the second much more slowly. The hydrogenated product melted at 135–145° and gave no color with hydrochloric acid.

Tetrahydrocafesteryl acetate (ox-cafestandiol-monoacetate) was prepared from the hydrogenated isocafesterol by treating it with acetic anhydride and pyridine in the usual manner. After crystallization it melted at 148–150°. A mixed m. p. with tetrahydrocafesteryl acetate, m. p. 146–148.5°, obtained by the hydrogenation of cafesteryl acetate, gave no depression.

(14) N. Levin, Thesis, Doctor of Philosophy, University of Maryland, 1941.

Anal. Calcd. for $C_{32}H_{54}O_4$: C, 72.89; H, 9.46. Found: C, 72.80; H, 9.18.

Isocafesterol Maleic Anhydride.—Isocafesterol (0.24 g., 0.0008 mole) was dissolved in 10 cc. of warm thiophene-free benzene and treated with 0.20 g. (0.0020 mole) of maleic anhydride similarly dissolved. Within thirty minutes a precipitate began to form. The solution was allowed to stand at room temperature overnight. The crystals were separated by filtration, washed with benzene and recrystallized from acetone; m. p. 177–180° (dec.); $[\alpha]^{30}D$ in acetone, $-45°$. The yield was 0.33 g. or 95% of the theoretical. A mixed m. p. with the starting material (147–158°) was 143–150°.

Anal. Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.60; H, 7.46.

A portion of the adduct was acetylated with acetic anhydride and pyridine in the usual manner. Several crystallizations from dilute acetone gave a product of m. p. 161–165.5°. Chemical analysis showed that during preparation and recrystallization decomposition had occurred with the formation of isocafesteryl acetate.

Anal. Calcd. for $C_{32}H_{50}O_4$: C, 73.71; H, 8.43. Found: C, 73.60; H, 8.18.

Decomposition of Isocafesterol Adduct.—Two hundred milligrams of isocafesterol maleic anhydride was decomposed at 0.01 mm. and 130–160°. The sublimate, a mixture of isocafesterol and maleic anhydride, was recrystallized from aqueous alcohol, giving isocafesterol, m. p. 145–152°. Treatment with hydrochloric acid produced the deep pink color characteristic of isocafesterol.

Anal. Calcd. for $C_{26}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.51; H, 8.65.

Spectrographic analysis of the sublimate and of a sample of isocafesterol gave identical curves (see Fig. 1) with no absorption in the region of 250–300 mμ.

A sample of **cafesterol maleic anhydride** was similarly decomposed at 0.008 mm. and 125–145°. The sublimate gave the blue color test for cafesterol[4] and spectrographic analysis showed a maximum at 292 mμ (see Fig. 1).

Summary

Cafesterol is isomerized, but not reduced, by the action of sodium and alcohol.

The new substance, isocafesterol, contains a reactive conjugated double bond system which is different from that present in cafesterol as indicated by absorption spectra data and other evidence.

KALAMAZOO, MICHIGAN RECEIVED APRIL 10, 1943