yield 6.5 g. (35%) of liquid acid IVb, b.p. 200–201° (0.2 mm.).[14]

Anal. Found: C, 72.91; H, 7.31.

Similar hydrolytic treatment of the liquid nitrile IIIb gave a 45% yield of the solid acid IVa, m.p. 153–155°, and 43% yield of the liquid acid IVb, b.p. 201° (0.2 mm.).[14]

3-Ethyl-5-methoxy-2-(*p*-methoxyphenyl)-1-indanone (VI).—According to the general method of Koo,[7] a mixture of 5.8 g. of solid acid IVa and 62 g. of polyphosphoric acid[15] was heated and stirred at 70° for 30 minutes. The reaction mixture was worked up as previously described[7] and 2.2 g. (39% yield) of the ketone VI, m.p. 90–92°, was obtained. Recrystallization of the sample from methanol–water yielded the pure compound VI, m.p. 92–94°.

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 76.87; H, 7.04.

The 2,4-dinitrophenylhydrazone of VI was prepared and recrystallized from chloroform–methanol, m.p. 186–187°.

Anal. Calcd. for $C_{25}H_{24}N_4O_6$: C, 63.01; H, 5.08. Found: C, 62.94; H, 5.31.

From the basic extract of the reaction just described, 2.5 g. (43%) of starting material IVa, m.p. 152–155°, was recovered through acidification.

1-Ethyl-6-methoxy-2(*p*-methoxyphenyl)-3-methylindene (V). (a) From the Nitrile IIIa.—The general procedure of Kohler[4a] was employed. To the Grignard reagent prepared from 12 g. of magnesium and 70 g. of methyl iodide in 150 ml. of anhydrous ether, a solution of 25 g. of the nitrile IIIa in 150 ml. of dry benzene was added slowly with stirring. After completion of the addition, the ether was removed and the resulting mixture heated at reflux temperature for three hours. After cooling, it was added slowly and with stirring to a mixture of 100 ml. of concentrated hydrochloric acid and cracked ice. After being allowed to reach room temperature to ensure complete hydrolysis of the Grignard complex, the mixture was extracted with ether containing a small amount of methanol, the ethereal solution was washed with saturated sodium chloride solution, dried further over sodium sulfate, and filtered. After removal of the ether, the residue distilled from a Hickman flask yielded 21 g. of an oil, b.p. 165–175° (0.3 mm.). The product was crystallized twice from alcohol to give 8 g. (32% yield) of needles, m.p. 97–99°. An analytical sample was prepared, m.p. 99–101°.

Anal. Calcd. for $C_{20}H_{22}O_2$: C, 81.60; H, 7.53. Found: C, 81.69; H, 7.70.

From the mother liquors no other compound could be identified. Treatment of the liquid nitrile IIIb under similar conditions led to a mixture, and no pure product could be isolated.

(b) From the Acid IVa.—The acid chloride was prepared from 6.3 g. of IV with 10 ml. of thionyl chloride under conditions previously employed.[16] The crude acid chloride (6 g.) was treated with diethyl ethoxymagnesium malonate[5] and the resulting substituted malonic ester was hydrolyzed and decarboxylated. Upon distillation of the product, 2 g. (32% yield) of an oil, b.p. 185–195° (0.5–1 mm.), was obtained. The compound was crystallized from acetone–Skellysolve C and was finally recrystallized from methanol to give a sample melting at 100–101°. Admixture of this compound with V, obtained from nitrile IIIa, did not depress the melting point.

(c) From the Ketone VI.—The ketone VI (3 g.) was allowed to react with methylmagnesium iodide[17] and the resulting crude carbinol was dehydrated by heating it at reflux temperature with 0.8 g. of *p*-toluenesulfonic acid in 40 ml. of acetic acid for 2.5 hours.[18] The crude reaction product, 2 g. (67%), crystallized after seeding it with a crystal of V obtained from the nitrile IIIa. The compound was recrystallized for analysis, m.p. 99–101°. Admixture of this compound with V, obtained from nitrile IIIa, did not depress the melting point.

Anal. Calcd. for $C_{20}H_{22}O_2$: C, 81.60; H, 7.53. Found: C, 81.64; H, 7.59.

(14) Experiment carried out by H. C. Scarborough, Jr.

(15) Obtained through the courtesy of Victor Chemical Works, Chicago 4, Ill.

(16) R. C. Fuson and J. T. Walker, ref. 13, Coll. Vol. II, 1943, p. 169.

(17) *Cf.* W. Voser, D. E. White, H. Heusser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **35**, 830 (1952).

(18) *Cf.* A. Eschenmoser, J. Schreiber and S. A. Julia, *ibid.*, **36**, 482 (1953).

LAWRENCE, KANS.

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

Studies in the Synthesis of the Antirachitic Vitamins. VII. The Synthesis of 2,1'-*cis* and 2,1'-*trans* Isomers of 1-Cholestanylidene-2-(5'-methoxy-2'-methylene-1'-cyclohexylidene)-ethane

BY NICHOLAS A. MILAS AND CHARLES P. PRIESING[1]

RECEIVED JULY 24, 1958

The synthesis of 2,1'-*cis*(VII) and 2,1'-*trans*(VIII) isomers of 1-cholestanylidene-2-(5'-methoxy-2'-methylene-1'-cyclohexylidene)-ethane, two homologs of vitamin D, from the corresponding isomeric dienones is described. During the purification of these homologs, a product was isolated which was chemically related to them but exhibited an abnormal ultraviolet spectrum. When tested on rachitic rats both the homologs and the third product were biologically active with the 2,1'-*cis*-homolog and the third product nearly as active as crystalline vitamin D_2, while the 2,1'-*trans*-homolog had very much less activity.

In connection with a long range program on the total synthesis of vitamin D_3 we had occasion to synthesize various homologs of this vitamin in order to study the influence of constitution on antirachitic activity. With the single exception of the calcium salt of the enol of 9,10-*seco*-cholest-5-en-7-one-3,10-diol[2] no biologically active antirachitic products of high activity have been obtained heretofore without the use of ultraviolet light or other high energy producing sources to activate the provitamin D intermediates which usually possess no antirachitic properties. It has already been established that high biological activity is associated with the stereochemical configuration of the triene system of the vitamin D molecule,[3a,b] and that the hydrocarbon side chain on carbon atom 17 is essential for this activity.[4] In view of the latter fact no attempt was made to assay biologically our simple homolog.[3b,5] However, in a recent communication[3a] we have announced the synthesis of a homo-

(1) From the Ph.D. Thesis of C. P. Priesing, M.I.T., April, 1957; presented before the 132nd Meeting of the A. C. S., New York, September 8–13 (1957).

(2) Y. Raoul, N. Le Boulch, C. Baron, R. Bazier and A. Gueriollot-Vinet, *Compt. rend.*, **242**, 3004 (1956).

(3) (a) N. A. Milas and C. P. Priesing, THIS JOURNAL, **79**, 3610 (1957); (b) **79**, 6295 (1957).

(4) N. A. Milas and R. C. Milone, *ibid.*, **68**, 738 (1946).

(5) N. A. Milas, L. C. Chiang, C. P. Priesing, A. A. Hyatt and J. Peters, *ibid.*, **77**, 4180 (1955).

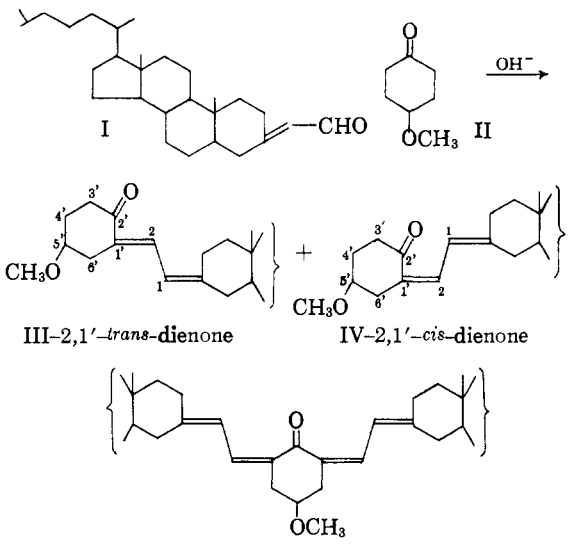


Fig. 1.—The aldol condensation of cholestanylidene acetaldehyde with 4-methoxycyclohexanone

log of vitamin D in which the unsubstituted cyclohexyl ring of the simple homolog was replaced by a cholestanyl moiety and the 2,1′-*cis* isomer of this homolog was found to approach the biological activity of the crystalline vitamin D_2. A more detailed account of this synthesis and that of the corresponding 2,1′-*trans*-isomer will be the subject of the present communication.

Discussion

It may be of interest to point out here that the 2,1′-*cis* homologs[3a,b] of vitamin D have been synthesized without photoisomerization of any of the intermediates[6] or of the final *trans* isomers.[7] We have already shown that our method of aldol condensation produces, in the case of the simple homolog, an equilibrium mixture of 2,1′-*cis* and 2,1′-*trans*-dienones which could not be separated easily. However, in the present case we have been able to effect the separation of the two isomers.

The synthesis of cholestanylidene acetaldehyde (I), the key intermediate in the present synthesis, has been described elsewhere.[8] In allowing this aldehyde to undergo an aldol condensation with 4-methoxycyclohexanone (II) under the same conditions as those described with the simple homolog,[3b] even after prolonged heating, no reaction was effected and the aldehyde was recovered unchanged. Since compounds in this series contain a conjugated system which does not emanate from a position adjacent to the ring junction, the aldehyde is not prone to undergo an *endo–exo* rearrangement and its stereochemical configuration will not be affected by alkali. In view of this fact the formation of the two stereoisomeric dienones must depend upon the mode of formation of the aldol and its subsequent dehydration. Since in 1,3-disubstituted cyclohexanes the groups are more stable in the equatorial conformations,[9] the initial attachment of the carbon atom containing the aldehyde group must also be equatorial.

The aldol condensation was carried out in *t*-butyl alcohol containing small quantities of water and alkali. If the dehydration of the aldol thus formed occurs in the presence of alkali, the 2,1′-*trans*-dienone III is produced. However, if the rate of dehydration is slow in *t*-butyl alcohol some of the aldol will undergo enolization and upon acidification may produce a different aldol which on dehydration will yield the 2,1′-*cis*-dienone IV. In the absence of more extensive data on the mechanism of the aldol condensation, the foregoing tentative explanation seems to account for the isolation of the two stereoisomeric dienones each of which itself is a diastereoisomeric mixture. In addition to the two stereoisomeric dienones, a small amount of the disubstitution product V was also isolated. The aldol condensation and the structure of the final products are illustrated in Fig. 1.

The aldol condensation yielded an ether-soluble (main product) and an ether-insoluble portion. The latter was identified as the disubstitution product V. When the ether was removed from the main fraction an amorphous yellowish-brown solid was obtained which had an ϵ value of 11,400 at 300 mμ. This product was chromatographed on alumina (Act. III) and eluted into eight fractions with solvents gradually increasing in polarity. Fractions 1–4 were identified, respectively, as self-condensation product of 4-methoxycyclohexanone (II), unchanged aldehyde I and small amounts of unidentified by-products.

Fraction 5 (main product) which was eluted with benzene showed the highest $E_{1\,cm}^{1\%}$ value at 302 mμ of 347 and was used for the source of both *cis*- and *trans*-dienones. Among the several methods tried to bring about a separation of the dienones, continuous extractions with hot ethanol resolved fraction 5 into ethanol-soluble and ethanol-insoluble portions. By repeated crystallization of the ethanol-soluble portion from ethanol at 0° two *trans* diastereoisomers were obtained: one, m.p. 133° with an ϵ(309 mμ) of 30600; the other m.p. 214–215°, with an ϵ(309 mμ) of 21000 in ethanol, respectively. The latter which was obtained in larger quantities showed three strong infrared bands for the dienone chromophore at 1674, 1614 and 1570 cm.$^{-1}$, respectively. It had also a high positive optical rotation, +86.8° (chloroform) and formed readily carbonyl derivatives which gave the expected analyses.

The ethanol-insoluble portion contained the *cis*-dienone which was obtained as a bright yellow amorphous solid, m.p. 186°, having an optical rotation of +40.3° (chloroform). The ultraviolet absorption spectrum of this dienone had a principal maximum at 307 mμ, ϵ 12200, a secondary maximum due to a partial chromophore at 233 mμ, ϵ 9670 and a shoulder at 340, ϵ 7400. The infrared spectrum showed bands for the dienone chromophore at 1675, 1615 and 1570 cm.$^{-1}$, respectively, with an additional band at 1708 cm.$^{-1}$ due to an isolated carbonyl.

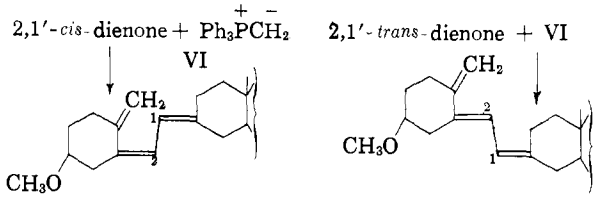
Unlike the *trans*-dienone the *cis*-dienone failed to give carbonyl derivatives due perhaps to steric ef-

(6) I. T. Harrison and B. Lythgoe, *Proc. Chem. Soc.*, 261 (1957).
(7) H. H. Inhoffen, G. Quinkert, H-J. Hess and H. Hirschfield, *Chem. Ber.*, **90**, 2544 (1957).
(8) N. A. Milas and C. P. Priesing, THIS JOURNAL, **80**, 2189 (1958).
(9) H. D. Orloff, *Chem. Revs.*, **54**, 355 (1954).

fects of proximate groups which block the carbonyl oxygen by inducing *S-cis–trans* isomerism and/or interference with uniplanarity.[10–12] This steric effect which may be brought about by a strong single bond twisting in the conjugated system of the *cis*-dienone is probably responsible for the lower extinction coefficient of its principal ultraviolet maximum and the appearance of a partial chromophore at 233 mμ. Moreover, the single bond twisting may also account for the appearance in the infrared spectrum of the isolated carbonyl absorption at higher frequencies.[13]

Fractions 6, 7 and 8 from the original chromatogram proved to be mixtures of 2,1′-*cis*-dienone and probably undehydrated aldol.

Additional evidence regarding the configuration of the two dienones was obtained when each dienone was allowed to react separately with triphenylphosphinemethylene VI (Wittig reagent). From the 2,1′-*cis*-dienone was obtained, after chromatography, a 23% yield of the 2,1′-*cis*-homolog VII as an amorphous, waxy product, m.p. 193° dec., λ_{max} 265 mμ, ε 20,200 (ether). Although this homolog was essentially pure at this stage, upon analysis it gave low carbon, so it was subjected to chromatography a second time, λ_{max} 267 mμ, ε 31,300 (ether), with an optical rotation of −10.6° (chloroform). The infrared spectrum of this homolog is compared in Fig. 1 with the infrared spectra of the 2,1′-*trans*-homolog and vitamin D_3.



VII, 2,1′-*cis*-homolog VIII, 2,1′-*trans*-homolog

From the 2,1′-*trans*-dienone was obtained, after chromatography, a 27% yield of the 2,1′-*trans* homolog VIII as an amorphous waxy product, λ_{max} 272 mμ, ε 34,700 (ether), with an optical rotation of −39° (chloroform).

During the purification of these isomers it was observed that, like natural vitamin D, they exhibited only limited stability. They are highly sensitive to light, air and heat and prolonged purifications caused a decrease in the carbon–hydrogen values, and a hypsochromic shift of the ultraviolet absorption maxima to the region 240–260 mμ, accompanied by lowering of the extinction coefficients.

In the original chromatography of both isomers the eluate with petroleum ether yielded a product which had a m.p. of 48.5–50° and a positive optical rotation of +7.44° (chloroform). Its ultraviolet absorption, however, was abnormal with ε(234 mμ) 13,300, ε(240 mμ) 13,600, ε(246 mμ) 13,900, ε(250 mμ) 13,500, ε(256 mμ) 11,400, and had no maxima in the expected region of vitamin D. The infrared spectrum was normal, comparing favorably with that of vitamin D_3 with the intensity of the bands for the exomethylene group exceptionally high. Moreover, this product showed normal analysis and upon microhydrogenation, it absorbed 3 mole-equivalents of hydrogen. The exact structure of this compound is not known at present and further work is needed for its elucidation.

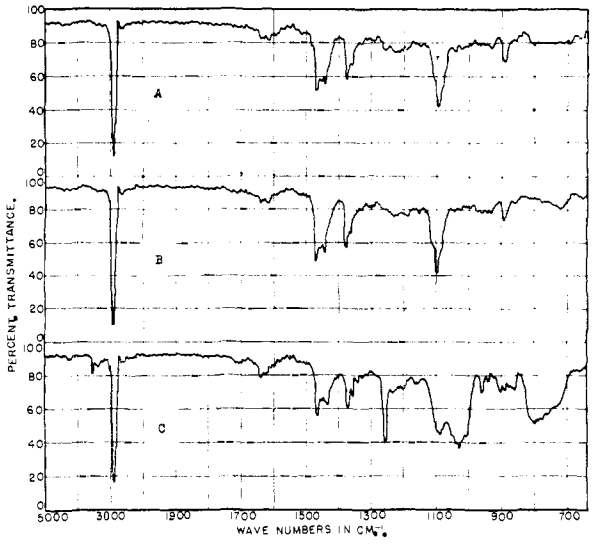


Fig. 1.—Infrared spectra (10% in chloroform) of A, 2,1′-*trans* homolog VII; B, 2,1′-*cis* homolog VIII; C, vitamin D_3.

Both the 2,1′-*cis* and 2,1′-*trans* homologs as well as the compound m.p. 48.5–50° were tested biologically on rachitic rats against crystalline vitamin D_2 by Professor Robert S. Harris of the Nutritional Biochemical Laboratories of M.I.T. The 2,1′-*cis* homolog and the compound m.p. 48.5–50° were found nearly as active as vitamin D_2 while the 2,1′-*trans* homolog was of much lower activity.

Experimental

Aldol Condensation of 2-Cholestanylideneëthan-1-al (I) with 4-Methoxycyclohexanone (II).—To a solution of 700 cc. of *t*-butyl alcohol containing 4 g. of sodium hydroxide and 10 cc. of water was added, dropwise in the course of one hour with stirring in an atmosphere of nitrogen, a mixture of 7.54 g. (0.0183 mole) of aldehyde I and 4.7 g. (0.0366 mole) of 4-methoxycyclohexanone (II) in 200 cc. of *t*-butyl alcohol. An additional 100 cc. of *t*-butyl alcohol was then added and the mixture stirred for 3 hours longer whereby it changed from pale-yellow to orange color. Stirring was continued in nitrogen at room temperature for 24 hours, then the mixture was cooled to 0° and treated with 200 cc. of iced-cold solution of tartaric acid containing 15 g. of the latter. The mixture was then extracted several times with ether and the ether extracts combined, washed once with water and dried over magnesium sulfate. The ether treatment of the reaction mixture caused the separation of a bright-yellow precipitate insoluble in both *t*-butyl alcohol and in ether. This was recrystallized from a chloroform–ether mixture; yield 0.5 g. (6%), m.p. 229.5–230°. The ultraviolet spectrum of this product showed two prominent bands: ε (248 mμ) 15,300, ε (365 mμ) 43,200 (in chloroform).

Anal. Calcd. for $C_{65}H_{104}O_2.H_2O$: C, 83.64; H, 11.43. Found: C, 83.66; H, 11.42.

(10) E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Chapter 4, Academic Press, Inc., New York, N. Y., 1955.

(11) L. L. Ingraham, "Steric Effects in Organic Chemistry," edited by M. S. Newman, John Wiley and Sons, Inc., New York, N. Y., 1956, p. 481.

(12) E. A. Braude and E. S. Waight, "Progress in Stereochemistry," edited by W. Klyne, Vol. 1, Chapter 4, Butterworth Scientific Pub., London, 1954.

(13) A. R. H. Cole, "Progress in the Chemistry of Organic Natural Products," edited by L. Zechmeister, Springer Verlag, Wein, Vol. 13, 1956, p. 1.

Repeated crystallizations and prolonged drying under high vacuum at elevated temperatures gave repeatedly analyses which were consistent for the monohydrate. An infrared spectrum of this ketone is recorded in Table I. On the basis of the foregoing results this ketone was assigned structure V.

When the dried ethereal solution from the aldol condensation was evaporated to dryness an amorphous yellowish-brown solid was obtained which had an ϵ (300 mμ) of 11,400. This product was dissolved in petroleum ether and chromatographed in the dark on a column of 200 g. of alumina (Act. III) and eluted with 400-cc. portions of solvents gradually increasing in polarity.

The product was resolved into eight fractions. Fraction 1 (1.3326 g.) was eluted with petroleum ether and proved to be a mixture of the self-condensation product of 4-methoxycyclohexanone II, and unchanged aldehyde I. Fraction 2 (0.0690 g.) was eluted with a 9:1 petroleum ether–benzene mixture and proved to be unchanged aldehyde. Fractions 3 and 4 which were eluted with 4:1 and 1:1 petroleum ether–benzene mixtures, respectively, were small amounts of unidentified products, and since they exhibited no absorption bands in the ultraviolet spectrum they were discarded.

Fraction 5 (main product, 2.0975 g.), which was eluted with benzene, was subjected to a continuous extraction with boiling ethanol which separated it into ethanol-soluble fraction (5A) and ethanol-insoluble fraction (5B). The ethanol-soluble fraction (0.9395 g., 10% yield) was recrystallized from absolute ethanol into pale-yellow needles (0.585 g.), m.p. 214–215° with softening at 200° and decomposition at 221–224°, $[\alpha]^{25}D$ + 86.8°, $[M]^{25}D$ + 45, 400 (chloroform). The ultraviolet spectrum of this ketone showed a maximum at 309 mμ, ϵ 21,000 (ethanol). The infrared spectrum of this dienone labeled as 2,1′-*trans*-dienone (III) is recorded in Table I.

TABLE I

INFRARED SPECTRA OF ALDOL CONDENSATION PRODUCTS (10% IN CARBON TETRACHLORIDE)

Compound	Group	Infrared bands, cm.$^{-1}$[a]
2,2,-*trans*-	—C=O	1674s, 1422rw, 1250s
Dienone (III)	R_2C=CHR	1614s, 1570s, 1290m, 890m, 863m
	—OCH_3	1310m, 1203w, 1190m, 1180r, 1140r, 1123r, 1110r, 1100s, 1080r, 1020r, 1042w, 1028w
	—C_6H_{20}	970m, 946m, 910m
2,1,-*cis*-Dienone	—C=O	1708m, 1675m, 1408r, 1250m, 1230m
(IV)	R_2C=CHR	1615s, 1570ms, 1290m, 890w, 860w
	—OCH_3	1310r, 1203w, 1190w, 1170r, 1140r, 1122r, 1112r, 1100s, 1080r, 1070r, 1025w
	—C_6H_{10}	970r, 942m, 903w
Disubstitution	—C=O	1670w, 1415r, 1250r, 1230s
product (V)	R_2C=CHR	1625vs, 1588s, 1290s, 890w, 864s
	—OCH_3	1310r, 1195w, 1180r, 1150m, 1125s, 1112r, 1100s, 1028w (in KBr)
	—C_6H_{10}	970r, 940s, 910w

[a] vs = very strong, s = strong, m = medium, w = weak, vw = very weak, r = shoulder.

Anal. Calcd. for $C_{36}H_{58}O_2$: C, 82.70; H, 11.18. Found: C, 82.73; H, 11.33.

The 2,4-dinitrophenylhydrazone of the dienone had a m.p. 184°.

Anal. Calcd. for $C_{42}H_{62}N_4O_5$: C, 71.76; H, 8.89; N, 7.97. Found: C, 71.56; H, 8.70; N, 8.56.

The semicarbazone had a m.p. 220–222°.

Anal. Calcd. for $C_{37}H_{62}N_3O_2$: C, 76.50; H, 10.76; N, 7.23. Found: C, 76.72; H, 10.95; N, 6.94.

When the mother liquor from the crystallization of the 2,1′-*trans*-dienone was cooled to −10° for several days a small amount of a colorless crystalline product separated out and was removed by filtration, washed with cold absolute ethanol and dried under high vacuum for 3 hours, m.p. 133°, ϵ (309 mμ) 30,300 (ethanol). Since this product was identical in every other respect with the 2,1′-*trans*-dienone it was concluded that it must be a diastereoisomer of the latter. When the last mother liquor was evaporated to dryness and the residue taken up in petroleum ether and once again flushed through a column of alumina, an additional 0.3 g. of 2,1′-*trans*-dienone was obtained.

The 5B fraction which was insoluble in hot ethanol could not be crystallized from any of the common solvents. It was further purified by flushing it with benzene through a column of alumina (Act. III) and removing the benzene under reduced pressure; yield 1.14 g. (12%) of amorphous solid, m.p. 186° with softening at 136°, $[\alpha]^{25}D$ + 40.3°, $[M]^{25}D$ +21,100 (chloroform).

Anal. Calcd. for $C_{36}H_{58}O_2$: C, 82.70; H, 11.18; $\overline{|}$, 2.00., Found: C, 82.71; H, 11.07; $\overline{|}$, 1.85 (Pd-on-C).

The ultraviolet spectrum showed a prominent band with an ϵ (307 mμ) of 12,200, a secondary band with an ϵ (233 mμ) of 9670 and a shoulder with an ϵ (233 mμ) of 7400 in ether, respectively. The infrared spectrum is recorded in Table I. Due to steric hindrance usually associated with the *cis*-dienones, all attempts to prepare a carbonyl derivative of this dienone met without success, and this fact together with its physical properties led us to conclude that this dienone has the 2,1′-*cis* configuration.

Fractions 6, 7 and 8 were eluted with ethyl ether, ethanol and glacial acetic acid, respectively. These fractions could not be purified by solvent partition, solvent extraction, crystallization or chemical separation. Each fraction, therefore, was separately flushed through a small column of alumina with its original solvent and the solution evaporated to dryness, the solid residue powdered in a mortar then subjected to a high vacuum at 80° for 24 hours. Fraction 6 (1.4514 g.) had a m.p. 155° (not sharp).

Anal. Calcd. for $C_{36}H_{58}O_2 \cdot H_2O$: C, 79.98; H, 11.19. Found: C, 80.31; H, 11.33.

The ultraviolet spectrum showed a prominent band with an ϵ (302 mμ) 14,300 (ether). The infrared spectrum showed bands, respectively, for –OH at 3500 (broad, strong) and 1024 cm.$^{-1}$ (weak); for —C=O at 1710(s), 1675(medium) and 1410(w) cm.$^{-1}$; for –OCH_3 at 1350(w) and 1100(vs); and for R_2C=CHR at 1616(m), 1645(w), 1570(m), 1295(w) and 860(w) cm.$^{-1}$. This product failed to form carbonyl derivatives. Fraction 7 (1.8531 g.) had a m.p. of 153° (not sharp).

Anal. Calcd. for $C_{36}H_{58}O_2.H_2O$: C, 79.98; H, 11.19. Found: C, 80.15, H, 11.41.

The ultraviolet spectrum of this fraction showed a prominent band with an ϵ (300 mμ) 8,800 (ether). The infrared spectrum showed essentially the same bands as that of fraction 6 except that there was a change in the intensity of most of the principal bands. Those remaining the same were the –OH bands; the strong band at 1030 cm.$^{-1}$ could be attributed to either –OH or –OCH_3. The —C=O band at 1712 cm.$^{-1}$ increased in intensity while that at 1665 cm.$^{-1}$ appeared as a shoulder. The band due to unsaturation at 1640 cm.$^{-1}$ had increased in intensity while those at 1615 and 1574 cm.$^{-1}$, respectively, were weak. The bands at 1290 and 860 cm.$^{-1}$ were also weak. The carbonyl group of this product was chemically inactive. Fraction 8 (0.8640 g.) had a m.p. of 157° (not sharp).

Anal. Calcd. for $C_{36}H_{58}O_2 \cdot H_2O$: C, 79.98; H, 11.19. Found: C, 79.66; H, 10.74.

The ultraviolet spectrum showed a prominent band with an ϵ (296 mμ) 4750 (ether). The infrared spectrum showed a shift in the –OH band to 3400 cm.$^{-1}$ with widening; the usual bands for –OCH_3 at 1330(w) and 1100(s) cm.$^{-1}$ and a much less intense band at 1022 cm.$^{-1}$. The —C=O band at 1708 cm.$^{-1}$ was very strong and wide while that at 1670 cm.$^{-1}$ appeared as a shoulder. The band for unsaturation at 1640 cm.$^{-1}$ was strong while those at 1616 and 1572 cm.$^{-1}$ appeared as shoulders.

2,1′-*cis* Isomer of 1-Cholestanylidene-2-(5′-methoxy-2′-methylene-1′-cyclohexylidene)-ethane (VII).—In a 300-cc. well-dried pressure bottle was placed 0.8 g. (2.2 mmoles) of methyl triphenylphosphonium bromide and to it was added in nitrogen 2.3 cc. of 0.8670 *N* phenyllithium (2.0 mmoles) in dry ether followed by a rapid addition of 50 cc. of dry ether. The bottle was stoppered securely and the mixture shaken at room temperature for 2 days in the dark whereby a bright yellow solution resulted. To this was then added in nitrogen 1.0 g. (2.0 mmoles) of 2,1′-*cis*-dienone IV, the bottle stoppered and heated in an oil-bath for 3 hours at 65–70°. The mixture was then cooled to room temperature and filtered in nitrogen through a sintered glass filter. In order to hydrolyze the excess reagents, the deep-red filtrate was washed twice with a saturated sodium chloride solution, thereby becoming yellow, dried over magne-

sium sulfate, filtered and the filtrate allowed to stand over nitrogen at −10° for 2 days. By filtering this mixture in the cold it was possible to remove all traces of triphenylphosphonium oxide.[14] Finally, the filtrate was evaporated under reduced pressure leaving a yellowish-brown viscous liquid; 0.9880 g. The ultraviolet spectrum of this crude product showed the maxima: $E^{1\%}_{1cm}$ (298 mμ) 153, (284 mμ) 146, (272 mμ) 132, (262 mμ) 138, (228 mμ) 140. This crude product was taken up in petroleum ether then adsorbed on alumina (Act. III) and eluted successively in nitrogen with 100-cc. portions of solvents according to the scheme: petroleum ether–benzene, 99:1, 49:1, 19:1, 9:1, 4:1, 1:1 and finally benzene. Each of these fractions was examined spectroscopically. The chromatographic elutions of petroleum ether–benzene, 19:1, 9:1 and 4:1 were combined and the solvent removed leaving a white amorphous solid, 0.2305 g. (23% yield). This solid had a m.p. of 193° dec. with softening at 160°. The ultraviolet spectrum had a principal maximum at 265 mμ with an ε 20,200 (ether). To purify this solid further, it was taken up in a mixture of petroleum ether–benzene 4:1 and flushed in nitrogen through a column of 3.0 g. of alumina. The solvent was evaporated and the white amorphous solid subjected to a high vacuum at 40–50° for 48 hours, $[\alpha]^{25}D$ −10.6, $[M]^{25}D$ −5520 (chloroform).

Anal. Calcd. for $C_{37}H_{60}O$: C, 85.32, H, 11.61. Found: C, 84.22; H, 11.55.

The principal prominent ultraviolet maximum of this homolog occurred at 267 mμ with an ε value of 31,200 (ether). Other maxima of much lower intensity occurred at 260, 264, 273 and 276 mμ, respectively. Like crystalline, vitamin D_3, this homolog gives with antimony trichloride, in chloroform a pink coloration which shows a prominent maximum at 514 mμ, $E^{1\%}_{1cm}$ 200.[15] The infrared absorption spectrum of this homolog is recorded in Fig. 1. When this homolog was tested biologically on rachitic rats it was found to be nearly as active as the crystalline vitamin D_2.

2,1′-*trans* Isomer of 1-Cholestanylidene-2-(5′-methoxy-2′-methylene-1′-cyclohexylidene)-ethane (VIII).—The 2,1′-*trans*-dienone III, m.p. 214–215°, was treated with triphenylphosphinemethylene (VI) exactly under the same conditions and concentrations as the 2,1′-*cis*-dienone IV, and after chromatographic treatment of the product formed, the eluent petroleum ether–benzene fractions 99:1 and 49:1 which had similar ultraviolet absorption maxima were combined and the solvent removed to give 0.2738 g. (27% yield) of a colorless gum, ε (272 mμ) 26,400 (ether). The gum was redissolved in 100 cc. of petroleum ether–benzene 49:1 mixture and flushed once in nitrogen through 3 g. of alumina. The solvent was then removed and the residue subjected to a vacuum at 40–50° for 48 hours and analyzed: $[\alpha]^{25}D$ −39.0°, $[M]^{25}D$ −20,400 (chloroform).

Anal. Calcd. for $C_{37}H_{60}O$: C, 85.32; H, 11.61. Found: C, 83.85; H, 11.34.

The principal prominent ultraviolet maximum of this homolog occurred at 272 mμ with an ε value of 34,700 (ether). Other maxima of much lower intensity occurred at 262, 265 and 277 mμ, respectively. With antimony trichloride in chloroform this homolog also gave a pink color which had an absorption maximum at 512 mμ, $E^{1\%}_{1cm}$ 159.[15] The infrared absorption spectrum is shown in Fig. 1. Like the *cis* homolog this was found to be highly unstable to heat, light and air.

When tested on rachitic rats this homolog was found to be very much less biologically active than either the 2,1′-*cis* homolog or crystalline vitamin D_2.

In the original chromatography of both isomers was eluted with petroleum ether a colorless semi-crystalline solid, m.p. 48.5–50°, $[\alpha]^{25}D$ + 7.44°, $[M]^{25}D$ + 38.0 (chloroform).

Anal. Calcd. for $C_{37}H_{60}O$: C, 85.32; H, 11.61. Found: C, 85.44; H, 12.47.

The ultraviolet spectrum of this product was abnormal; it showed maxima with ε(234 mμ) 13,300, ε(240 mμ) 13,600, ε(246 mμ) 13,900, ε(250 mμ) 13,500, ε(256 mμ) 11,400. The infrared spectrum (10% in chloroform) showed the following bands, respectively, for the groups: $=CH_2$, 3100(r), 1648(s), 885(s) cm.$^{-1}$; $R_2C=CHR$, 1630(r), 1598(vw), 1300(w), 860(r) cm.$^{-1}$; $-OCH_3$, 1315(vw), 1260(m), 1182-(w), 1157(w), 1143(w), 1134(w), 1115(r), 1090(m), 1020(r), 1024(m), 1008(r) cm.$^{-1}$; $-C_6H_{10}$, 973(r), 960(w), 943(w), 930(r), 922(w), 902(r) cm.$^{-1}$. With antimony trichloride in chloroform this fraction gave only a slight pink coloration with an $E^{1\%}_{1cm}$ (510 mμ) 38.4. Microhydrogenation in ethyl acetate using palladium-on-charcoal absorbed 3.0 mole-equivalents of hydrogen showing the presence of 3 double bonds.

In spite of the abnormal ultraviolet spectrum exhibited by this fraction it was found to be as active biologically as the 2,1′-*cis*-homolog when tested on rachitic rats.

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(14) Care must be taken to remove all traces of triphenylphosphonium oxide since its ultraviolet absorption spectrum shows a maximum at 266 mμ with an $E^{1\%}_{1cm}$ of 77 and could easily be mistaken for the vitamin D maximum at 265 mμ.

(15) Since this homolog is highly sensitive to air oxidation and no special precautions were taken to exclude air during measurements the value given is low.

[CONTRIBUTION FROM THE FISHERIES RESEARCH BOARD OF CANADA, CHEMISTRY SECTION OF THE TECHNOLOGICAL STATION AT VANCOUVER]

Marine Sterols. V. Isolation of 7,24(28)-Ergostadien-3β-ol from Starfish[1]

BY U. H. M. FAGERLUND AND D. R. IDLER

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A new sterol, 7,24(28)-ergostadien-3β-ol, has been isolated by a chromatographic separation of the azoyl esters prepared from the sterols of the starfish *Pisaster ochraceus*. Starfish sterols contained 19% of this sterol.

The sterols of starfish are generally conceded to be entirely of the C_7-unsaturated type and 7-cholestenol,[2–4] previously found in mammalian skin,[5] recently has been isolated. Hitodesterol has been shown to be identical with α-spinasterol (7,22-stigmastadienol).[6–8] 7-Stigmastenol, a recog-

(1) Presented at the Portland Meeting of the American Chemical Society, June, 1958.

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