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Chemistry of Vitamin A. XXV. Geometrical Isomers of Vitamin A Aldehyde and an Isomer of its α-Ionone Analog[1]

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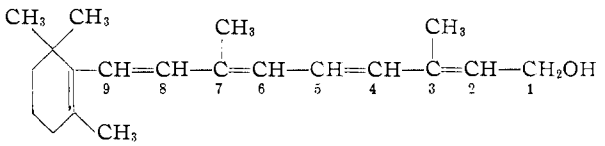
The preparation of four, synthetic geometrical isomers of vitamin A aldehyde (identified as all-*trans*, 2-*cis*, 2,6-di-*cis*, 6-*cis*) is described. Their ultraviolet absorption spectra, infrared spectra and other physical and chemical properties are given. The configuration of a fifth isomer of vitamin A aldehyde, neoretinene-b, was also studied. A 2,4-di-*cis* structure was preferred for it over a possible 2-*trans*, 4-*cis* configuration. An all-*trans* "α-vitamin A aldehyde" was synthesized from α-ionone. Its properties were different from those of the 6-*cis* and 2,6-di-*cis* isomers which excluded the possibility that the latter two compounds might be geometrical isomers of α-vitamin A aldehyde.

In the previous paper in this series[2] a synthesis of vitamin A is described by which four crystalline geometrical isomers were prepared. These were identified as the all-*trans*, 2-*cis*, 6-*cis* and 2,6-di-*cis* forms, according to the following numbering system



Similar reactions were employed to synthesize an "α-vitamin A" in which the ring double bond was shifted out of conjugation with the side chain, as in α-ionone.

After oxidation with manganese dioxide,[3] the corresponding crystalline vitamin A aldehydes were prepared, together with a number of derivatives. Some of the properties of these compounds are given in Table I.

These properties in conjunction with published properties on three isomers, assigned the all-*trans*, 2-*cis*, 6-*cis* configurations (Table I, footnotes) supported the structural assignments made in reference 2 and indicated that no change in configuration had occurred during oxidation. The 2,6-di-*cis* isomer has not been described previously and its assigned structure was based on that assigned the parent vitamin A as well as on confirmatory evidence obtained from infrared data presented later in this paper.

The α-vitamin A aldehyde isomer we prepared has also not been described before. It was assigned an all-*trans* configuration, partly because of its failure to isomerize with acid reagents to an isomer absorbing at a longer wave length and because of its infrared spectrum.

TABLE I

PROPERTIES OF ISOMERS AND DERIVATIVES OF VITAMIN A ALDEHYDE

Isomer or derivative	M.p., °C.	Absorption[a] λmax, mμ	$E^{1\%}_{1\,cm}$	ε	$SbCl_3$ reaction product λmax, mμ	$E^{1\%}_{1\,cm}$
All-*trans*[c]	57, 65[b]	381	1530	43,400	664	3470
Methyl nicotinium *p*-toluenesulfonate hydrazone	192.5	395	892	52,500		
-2,4-dinitrophenylhydrazone (DNPH)	211	263, 350, 448	373, 441, 1115	—, —, 51,700 ($CHCl_3$)		
-sulfanilamide Schiff base	162	403	1335	58,500		
-*p*-aminoazobenzene Schiff base	138.5	422	1340	62,200		
Neo- (2-*cis*, neoretinene-a)[d]	77	257, 375	336, 1250	—, 35,600	664	3490
2,6-di-*cis* (isoretinene-b)	49, 85[b]	368	1140	32,400	664	3490
-DNPH derivative	209	425	1020	47,300 ($CHCl_2$)		
6-*cis* ("*cis*-" vitamin A aldehyde, isoretinene-a)[e]	64	373	1270	36,100	664	3470
-DNPH derivative	189	438	1070	49,700		
-semicarbazone	192	294, 362, 373	336, 2110, 2120	—, —, 72,300		
Neoretinene-b (2,4-di-*cis*-vitamin A aldehyde)[f]	64.5	254, 376	595, 857	16,950, 24,400	664	3498
α-Vitamin A aldehyde (all-*trans*)	85.5	250, 368	284, 1720	—, 48,800	561	4420
-semicarbazone	195	343, 358, 378	1630, 2580, 2350	—, 88,000,—		
-DNPH derivative	185	255, 350, 429	513, 391, 1100	—, —, 51,100 ($CHCl_3$)		

[a] In absolute ethanol unless otherwise specified. [b] Dimorphic forms. [c] All-*trans*-vitamin A aldehyde from natural vitamin A (m.p. 61–62°, $E^{1\%}_{1\,cm}$ 1 cm.)(385.5 mμ) 1400[3]). From all-*trans* synthetic vitamin A (m.p. 61–62°, $E^{1\%}_{1\,cm}$ 1 cm.) (383 mμ) 1600[4]). [d] Neovitamin A aldehyde from natural neovitamin A (neoretinene a, m.p. 75°, $E^{1\%}_{1\,cm}$ 1 cm.) (377 mμ) 1190[4]). [e] "*cis*" (6-*cis*)-vitamin A aldehyde (m.p. 60°, $E^{1\%}_{1\,cm}$ 1cm.)(373 mμ) 930[5]). The two parent vitamin A alcohols were likewise similar in properties (ours, m.p. 82°, theirs, m.p. 85.3°). [f] Dieterle and Robeson.[6] A few crystals were prepared previously by Hubbard and Wald,[7] having $E^{1\%}_{1\,cm}$ 1cm.)(377.5 mμ) 900 to 1000.

(1) Communication No. 206. Presented in part before the Division of Biological Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.

(2) C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger and A. J. Chechak, THIS JOURNAL, **77**, 4111 (1955).

(3) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, No. 4, 516 (1948).

(4) R. Hubbard, R. I. Gregerman and G. Wald, *J. Gen. Physiol.*, **36**, No. 3, 415 (1953).

(5) W. Graham, D. A. van Dorp and J. F. Arens, *Rec. trav. chim. Pays-Bas*, **68**, 609 (1949).

(6) J. M. Dieterle and C. D. Robeson, *Science*, **120**, 219 (1954).

(7) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952–1953).

Dimorphic forms of the all-*trans* vitamin A aldehyde isomer were prepared following the finding that the melting point of our early preparations (55.5–57°) was lower than the value of 62°.[3] Professor Wald kindly furnished seed crystals and we then obtained the higher melting form. After repeated crystallization from petroleum ether, this had m.p. 64–65°. Dimorphic forms of the 2,6-di-*cis* isomer were also prepared (Table I).

Ultraviolet Absorption Spectra.—The ultraviolet absorption curves for the aldehyde isomers and for a fifth vitamin A aldehyde isomer, neoretinene-b, are given in Fig. 1.[8] Neoretinene-b is the isomer which Hubbard and Wald have identified, from *in vitro* experiments, as the precursor of the visual pigment rhodopsin.[7] This isomer was later studied in this Laboratory.[6]

The position of the main absorption maximum for the vitamin A aldehyde isomers was shifted to shorter wave lengths, as expected, with the increase in the number of *cis*-double bonds. The spectrum for the α-vitamin A aldehyde isomer was of interest in that it had a single peak in the region of maximum absorption (368 mμ) as compared to the triple peak for α-vitamin A alcohol[2] and α-vitamin A methyl ether.[9]

Similarities in the absorption curves divided the vitamin A aldehyde isomers as follows: (1) the all-*trans* (highest extinction coefficient at main peak, small subsidiary peak, 245 mμ), (2) neoretinene-b and neovitamin A aldehyde (marked subsidiary peaks, 255, 258 mμ), (3) the 6-*cis* and 2,6-di-*cis* isomers, faint subsidiary peaks, 250, 253 mμ; flex points at 295 mμ). The position of the "*cis* peaks" as defined by Zechmeister and Polgár[10] is uncertain. They may be in the 250–260 mμ region, but the variable position of the maximum distinguished these isomers from those of the carotenoids which Zechmeister and his coworkers have studied. This variation in maximum merits further study.

Antimony Trichloride Reaction.—The absorption coefficients of the blue color formed by reaction of the six aldehydes with antimony trichloride are given in Table I (method, Experimental Part). Values for all but the α-vitamin A aldehyde isomer have been previously reported by Hubbard, *et al.*,[4] using in part preparations from these laboratories.

Like these workers, we found that all five vitamin A aldehyde isomers gave blue colors with similar absorption curves and extinction coefficients suggesting that the product(s) of the reaction has the same geometrical configuration. Our values for the extinction coefficients were about 92% of those reported in the reference.

The color formed with α-vitamin A aldehyde was red (λ_{max} 561 mμ), which distinguishes this aldehyde from the vitamin A aldehyde isomers. The possibility that our 6-*cis* and 2,6-di-*cis* aldehydes might be geometric isomers of α-vitamin A aldehyde is thus excluded. This possibility received some consideration[4] because certain theoretical objections appeared to exist at that time to there being five geometric isomers of vitamin A aldehyde. The existence of these five forms is now established.

(8) Mr. A. P. Besancon and assistants of these laboratories made the measurements using a Cary recording spectrophotometer (Model 11 M).

(9) W. Oroshnik, THIS JOURNAL, **76**, 5499 (1954).

(10) L. Zechmeister and A. Polgár, *ibid.*, **65**, 1522 (1943).

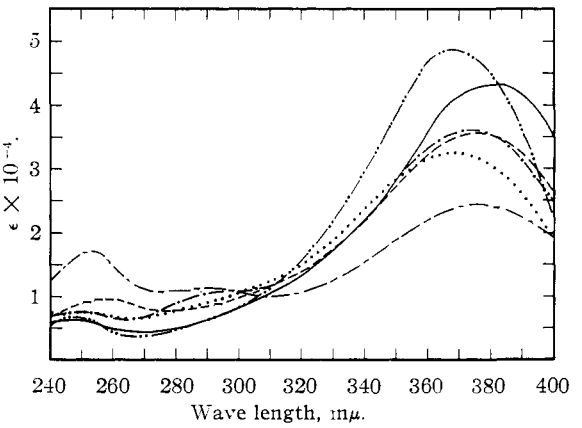


Fig. 1.—Ultraviolet spectra of vitamin A aldehyde isomers: all-*trans* (—); neo- (------); 2,6-di-*cis* (. . . . .); 6-*cis* (-·-·-·-); α- (-···-···-); neoretinene-b (— –).

A sample of α-vitamin A aldehyde was also submitted to Prof. Wald for examination by the opsin method. It failed to form a light sensitive pigment, either before or after irradiation. This behavior serves further to distinguish α-vitamin A aldehyde from the vitamin A aldehyde isomers.

Infrared Spectra and Structure of Neoretinene-b.—The infrared curves of the isomers are given in Fig. 2 (method, Experimental Part). In Table II are given the wave lengths and extinction coefficients (k) for the C=O stretching band.

TABLE II

INFRARED CURVES: WAVE LENGTHS AND EXTINCTION COEFFICIENTS FOR C=O BANDS OF ISOMERS

Vitamin A aldehyde isomer	Wave length C=O, μ	k C=O	Vitamin A aldehyde isomer	Wave length C=O, μ	k C=O
All-*trans*	6.01	3.68	Neo-	5.99	2.85
All-*trans* α-	6.01	4.16	2,6-di-*cis*	5.99	2.84
6-*cis*	6.01	3.44	Neoretinene-b	5.99	2.55

The data are consistent with the postulated structures for the first five isomers in the table on the reasonable assumption that the configuration in the 2-position is of major importance in determining the position of the carbonyl band. On this basis, the all-*trans*, 6-*cis* and α-vitamin A isomers fall into a 2-*trans* class, while the neo-, 2,6-di-*cis* and neoretinene-b isomers fall into a 2-*cis* class.

The extinction coefficients of the 2-*trans* compounds are also higher than for the 2-*cis* isomers, but the value varies with other properties of the molecule. The extinction coefficient for α-vitamin A aldehyde is higher than for all-*trans* vitamin A aldehyde, probably because of the steric hindrance between the ring and side chain present in the latter compound.[9] From the data, neoretinene b would appear to have a 2-*cis* structure and greater "*cis*-ness" than the other isomers as indicated by the lower absorbency at 5.99 μ.

Infrared measurements at other wave lengths appeared to link neoretinene-b with the 2-*cis*,

TABLE III

WAVE LENGTHS AND EXTINCTION COEFFICIENTS FOR ISOMERS AT OTHER PEAKS

Vitamin A aldehyde isomer	k at μ indicated				
All-*trans*	0.17 (12.2)	0.065 (12.93)	0.46 (7.52)	0.74 (9.02)	No peak
All-*trans* α-	.37 (12.18)	.08 (12.98)	.44 (7.52)	.90 (9.00)	No peak
6-*cis*	.19 (12.22)	.08 (12.96)	.40 (7.52)	.77 (9.02)	No peak
2-*cis*	No peak	No peak	No peak	1.15 (8.99)	0.12 (13.22)
2,6-Di-*cis*	No peak	No peak	No peak	1.25 (8.99)	.12 (13.23)
Neoretinene-b	No peak	No peak	No peak	0.70 (8.93)	.17 (13.21)

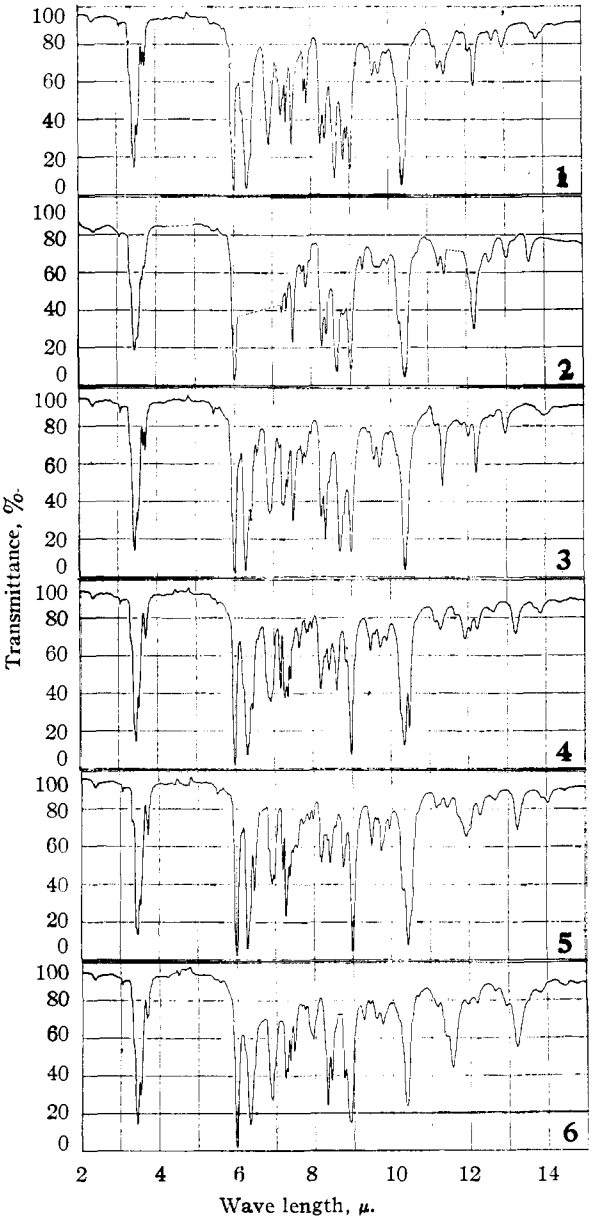


Fig. 2.—Infrared spectra of vitamin A aldehyde isomers: (1) all-*trans*; (2) α-; (3) 6-*cis*; (4) 2-*cis*; (5) 2,6-di-*cis*; (6) neoretinene-b.

rather than with the 2-*trans* isomers, although our present lack of knowledge of the origin of these peaks makes the data of interest, rather than of value as evidence, at this time (Table III).

The absorption data for the band at 10.3–10.4 mμ are given in Table IV.

TABLE IV

WAVE LENGTHS AND EXTINCTION COEFFICIENTS AT 10.3–10.4 μ FOR ISOMERS

Vitamin A aldehyde isomer	Wave length, μ	k	No. of "unsubstituted" *trans*-double bonds
All-*trans*	10.36	1.57	2
All-*trans*-α-	10.37	1.51	2
6-*cis*	10.40	1.52	2
2-*cis*	10.36	1.06	2
2,6-Di-*cis*	10.41	1.07	2
Neoretinene-b	10.37	0.58	1

The strong band at about 10.4 mμ corresponds to the out-of-plane hydrogen bending vibration of a *trans*, —CH=CH—, or "unsubstituted *trans*" bond, and its intensity increases with the number of such bonds in a conjugated system.[11–15] The extinction coefficients (Table IV) are consistent with five of the isomers having two unsubstituted *trans* bonds, as postulated, the varying coefficients corresponding to a varying number of *cis* "substituted," —C(CH$_3$)=CH— bonds.

The substantially lower extinction coefficient for neoretinene-b suggests that it has only one unsubstituted *trans* bond, the *cis*-unsubstituted bond being in either the 4- or 8-position. Models show, however, that a *cis* bond in position 8 would cause such a break in coplanarity between ring and side chain as to virtually exclude the ring double bond from affecting the ultraviolet absorption maximum of the conjugated system. Such a molecule might be expected to absorb at a wave length approximating that of the corresponding α-vitamin A aldehyde isomer and probably not exceeding 368 mμ, the value for the all-*trans* α-isomer. The higher absorption maximum found for neoretinene-b (375 mμ) therefore suggests that the more probable position for the *cis* bond is at position 4 and neoretinene-b is therefore tentatively assigned a 2,4-di-*cis* structure.

The possibility that neoretinene-b has an 8-mono-*cis* configuration was further examined by treating the corresponding vitamin A isomer (neovitamin Ab,[7] prepared from the aldehyde by reduction with lithium aluminum hydride[6]) with maleic anhydride under controlled conditions.

(11) R. S. Rasmussen, R. R. Brattain and P. S. Zucco, *J. Chem. Phys.*, **15**, 135 (1947).

(12) O. D. Shreve, M. R. Heether, H. B. Knight and D. Swern, *Anal. Chem.*, **22**, 1261 (1950).

(13) J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **29**, 229 (1952).

(14) R. F. Paschke, W. Tolberg and D. H. Wheeler, *ibid.*, **30**, 97 (1953).

(15) L. Zechmeister, *Experientia*, *X*, 1 (1954).

The addition reaction involves the 2- and 4-double bonds and hence proceeds rapidly only when these bonds are *trans*-.[2,23] The reaction rate with the vitamin A isomer from neoretinene-b was slow (apparent "neovitamin A" content = 74% by formula of ref. 23), ruling out the 8-mono-*cis* configuration on the basis of our work.

The 74% figure indicates that the rate of reaction was somewhat faster than with neovitamin A. This may have been due to the presence of some all-*trans*-vitamin A in the neovitamin Ab preparation, produced during the reduction reaction. Alternatively, some all-*trans*-vitamin A may have been formed by catalytic action of the maleic anhydride.

A *cis* bond in the 4-position represents a "hindered *cis*" structure[16] whose possible existence has been questioned. A number of compounds having hindered *cis* double bonds have, however, been prepared in recent years, some by catalytic reduction of the corresponding compound containing a triple bond,[17] some by other synthetic means.[18] Another method of preparing such compounds is by direct introduction of energy by illuminating an all-*trans* polyene, the method used to make neoretinene b.

Although the assignment of a 4-*cis* configuration for neoretinene-b seems reasonably certain, private correspondence with Prof. Wald and Miss Ruth Hubbard has indicated that the 2-*cis* structure can be challenged. Experiments conducted in Prof. Wald's laboratory, including experiments in which neoretinene-b was subjected to thermal isomerization and gave all-*trans* vitamin A aldehyde, were interpreted by Wald and Hubbard to indicate a 2-*trans* configuration.[19] Isomerization experiments conducted in our laboratory, and discussed in the next section, also indicated that upon iodine isomerization in the dark, neoretinene-b gave all-*trans*-vitamin A aldehyde as the first detectable product.

We consider this evidence insufficient, as yet, to outweigh the conclusion drawn on the basis of infrared studies. A number of *cis* carotenoids (*e.g.*, cryptoxanthin),[20] when isomerized with iodine, have yielded predominantly the all-*trans* form. Certain polyenes with hindered *cis*-double bonds (*cis–trans* or *cis–cis*-diphenylbutadiene) gave exclusively the all-*trans* compound upon isomerization with iodine,[21] provided light was present. These considerations plus some suggestive evidence from bioassays[22] have caused us, at present, to consider the 2,4-di-*cis* structure for neoretinene-b to be the more probable one.

(16) L. Pauling, *Fortschr. Chem. organ. Naturstoffe*, **3**, 203 (1939); L. Zechmeister, *Chem. Revs.*, **34**, 267 (1944).

(17) W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 295 (1952); C. F. Garbers, C. H. Eugster and P. Karrer, *Helv. Chim. Acta*, **35**, 1850 (1952); **36**, 562 (1953); C. F. Garbers and P. Karrer, *ibid.*, **36**, 828 (1953); W. Oroshnik and A. D. Mebane, THIS JOURNAL, **76**, 5719 (1954).

(18) Evidence to support 4-*cis* configurations for vitamin A diacid isomers and other polyene diacids and monoacids is provided in two other papers from these laboratories (J. D. Cawley, *ibid.*, **77**, 4125 (1955); J. D. Cawley and D. R. Nelan, *ibid.*, **77**, 4130 (1955).

(19) Private communication, Miss Ruth Hubbard.

(20) L. Zechmeister and R. M. Lemmon, THIS JOURNAL, **66**, 317 (1944).

(21) J. H. Pinckard, B. Wille and L. Zechmeister, *ibid.*, **70**, 1938 (1948).

(22) S. R. Ames, W. J. Swanson and P. L. Harris, *ibid.*, **77**, 4134 (1955).

Isomerization.—Isomerization experiments indicated that interconversion of the vitamin A aldehyde isomers proceeds with relative ease. Treatment of the all-*trans*, neo-, 6-*cis* or 2,6-di-*cis* aldehydes with acidic reagents, *e.g.*, pyridine hydrochloride, in the absence of light, led to mixtures of isomers in which all but the 2,6-di-*cis* compound could be identified by infrared analysis. Isomerization was accompanied by a shift in the position in the absorption maximum, and a change in the absorption coefficient, up or down, depending on the isomer used. An infrared technique has not yet been worked out for determining accurately the composition of mixtures containing more than two isomers, but from the curves for the individual pure isomers, it was established that the all-*trans* compound predominated in all the mixtures.

Isomerization of the all-*trans* isomer with iodine, in benzene solution, in the dark led to a mixture which, at equilibrium, contained the all-*trans* and neo-isomers in the approximate ratio 70:30, by infrared analysis. This was the expected result. When the isomerization was conducted in diffused sunlight, the reaction was more complicated and some of the 6-*cis* isomer was formed. The all-*trans* compound constituted about 55% of the mixed isomers.

Isomerization of 2,6-di-*cis*-vitamin A aldehyde for 2 hours in the dark, with iodine, gave 25% of the 6-*cis* isomer plus unchanged 2,6-di-*cis*. Inasmuch as 70% of the 6-*cis* isomer was expected to be present at equilibrium, the interconversion appeared to proceed slowly under the experimental conditions. In the presence of diffused light, a mixture containing approximately equal amounts of the 6-*cis*, 2,6-di-*cis* and all-*trans* isomers was formed.

Neoretinene-b, after isomerization with iodine for 2 hours, in diffused light, gave a mixture containing about 55% of the all-*trans* isomer plus smaller amounts of the 2-*cis* and 6-*cis* isomers. When the reaction time was 5 or 15 minutes in the dark, neoretinene-b and the all-*trans* isomer were the only products detected. Thus the primary isomerization product of neoretinene-b, with iodine, is the all-*trans* isomer. The analytical method might well have failed to detect small amounts of other *cis* isomers, however.

α-Vitamin A aldehyde showed no evidence of isomerization, as evidenced by a significant change in the extinction coefficient or the position of the ultraviolet absorption maximum, when treated with pyridine hydrochloride under conditions which isomerized the vitamin A aldehyde isomers. Interpretation of a small shift of about 5 mμ in the absorption maximum after a longer reaction time was complicated by a substantial, concomitant loss in absorption. It was concluded that this isomer isomerizes with greater difficulty than the vitamin A aldehyde isomers, under these conditions.

Experimental Part

Melting points were determined with a capillary tube and 3 inch immersion thermometer.

Preparation of Isomers

Five of the isomers (all-*trans*, neo-, 6-*cis*, 2,6-di-*cis* and α-vitamin A aldehydes) were prepared from the corresponding alcohols by oxidation with manganese dioxide according to a procedure which was similar to that described by Ball, *et al.*[3] The method is illustrated by the preparation of the 2,6-di-*cis*-isomer.

To a solution of 2,6-di-*cis*-vitamin A (2 g., *E*(1%, 1 cm.) (324 mμ) 1260) in petroleum ether (20 ml., Skellysolve F) was added activated manganese dioxide (14 g., "precipitated," General Metallic Oxides Co.), portionwise, with stirring. After standing for 20 hours at room temperature, protected from the light, the reaction mixture was adsorbed on sodium aluminum silicate (25 g., Doucil, Philadelphia Quartz Co.) that had been previously washed in succession with acetone (50 ml.) and petroleum ether to remove solubles. The column was washed with petroleum ether and the filtrate was evaporated under nitrogen.

The resulting crude aldehyde (1.46 g., *E*(1%, 1 cm.) (368 mμ) 1020) was crystallized from petroleum ether (5 ml.) at −25°. After 48 hours the yellow crystals were filtered on a cold Buchner funnel. After washing with a small amount of cold petroleum ether the crystals were dried under vacuum (0.6 g., m.p. 47–49°, *E*(1%, 1 cm.) (368 mμ) 1100). A second crop of crystals (0.6 g.) was obtained by concentration of the mother liquor and crystallization at −25° (m.p. 47–49°, *E*(1%, 1 cm.)(368 mμ) 1140, over-all yield from vitamin A isomer, 68%, by ultraviolet absorption data).

On recrystallization from petroleum ether, this last preparation appeared to become less soluble and required more solvent to bring it into solution. On cooling, crystals of the other form were obtained, m.p. 84–85°, *E*(1%, 1 cm.) (368 mμ) 1140.

Yields of vitamin A aldehyde isomer from the corresponding vitamin A isomer for the steps (1) oxidation plus chromatographic purification and (2) crystallization were as follows: all-*trans* (73, 80%), neo- (70, 79%), 6-*cis* (77, 61%). The yield of crystalline all-*trans*-α-vitamin A aldehyde (0.6 g.) from mixed isomer concentrate[2] (2.5 g.) was poorer.

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: isomers, all-*trans:* C, 84.5; H, 9.7. 2-*cis:* C, 84.7; H, 9.7. 6-*cis:* C, 84.6; H, 9.7. 2,6-di-*cis:* C, 84.8; H, 9.7. α-Vitamin A aldehyde: C, 83.3; H, 10.0.

Preparation of Derivatives. Properties, Table I

All-*trans* **Vitamin A Aldehyde.** (a) **Methylnicotinium *p*-Toluenesulfonate Hydrazone.**—To all-*trans*-vitamin A aldehyde (1.45 g.) and methylnicotinium *p*-toluenesulfonate hydrazide (1.58 g.) was added ethanol (10 ml.). The mixture was heated for 5 minutes to effect solution and then allowed to stand for 2 hours at room temperature. The yellow-orange crystals were collected by suction on a Buchner funnel, washed with a little ethanol and dried under vacuum (2.5 g.).

Anal. Calcd. for $C_{34}H_{43}O_4N_3S$: C, 69.23; H, 7.35; N, 7.12. Found: C, 69.7; H, 7.2; N, 6.8.

(b) **2,4-Dinitrophenylhydrazone.**—(Same procedure applied to other isomers.) The aldehyde (1.0 g.) was dissolved in ethanol (26 ml.) and 2,4-dinitrophenylhydrazine (0.70 g.) was added. After bringing to reflux, concentrated hydrochloric acid (0.95 ml.) was added and the mixture refluxed for 15 minutes. The reaction mixture was cooled and filtered immediately to inhibit isomerization.

The crude product was crystallized three times from ethyl acetate, reddish-purple needles. The melting point (211°) was similar to that reported by Ball, *et al.*[3]

(c) **Sulfanilamide Schiff Base.**—To the aldehyde (2.84 g.) in warm methanol (10 ml.) was added a solution of sulfanilamide (1.72 g.) in warm methanol (10 ml.). The mixture was refluxed for 30 minutes and allowed to crystallize for 2 hours. The orange crystals were collected by suction, washed with methanol and petroleum ether and dried under vacuum (0.9 g.).

A second crop of Schiff base (2.63 g.) was obtained by refluxing the filtrate for an additional 30 minutes, concentrating it to 15 ml., and crystallizing as before.

Anal. Calcd. for $C_{26}H_{34}O_2N_2S$: C, 71.19; H, 7.81; N, 6.39. Found: C, 71.0; H, 8.0; N, 6.1.

(d) **Aminoazobenzene Schiff Base.**—A mixture of aldehyde (0.6 g.) and *p*-aminoazobenzene (0.4 g.) was heated with methanol (4 ml.) to effect solution. Orange-red crystals separated on standing for 20 hours at room temperature. These were collected by suction, washed with a little methanol and dried under vacuum (0.9 g.).

Anal. Calcd. for $C_{32}H_{37}N_3$: C, 82.89; H, 8.04; N, 9.06. Found: C, 82.1; H, 8.1; N, 9.1.

6-*cis*-Vitamin A Aldehyde. Semicarbazone.—Procedure also was used for α-vitamin A aldehyde. A mixture of the aldehyde (0.15 g.), sodium acetate (0.22 g.), semicarbazide hydrochloride (0.15 g.) and ethanol (1.5 ml.) was treated, dropwise, with just enough water to dissolve the salts. After refluxing for 30 minutes the solution was cooled to room temperature. The crystals which separated were recrystallized from ethanol. The melting point we found (192°) was lower than that reported by Graham, *et al.* (195°).[5]

Anal. Calcd. for $C_{21}H_{31}ON_3$: C, 73.85; H, 9.15; N, 12.30. Found: C, 72.9; H, 9.1; N, 12.4.

Infrared Measurements.—All samples were run in solution so that any effects due to polymorphic forms were eliminated. The data in Fig. 2, except for the 6.1–7 μ region, were obtained with a Perkin–Elmer, double beam instrument (Model 21), with sodium chloride prism and with carbon disulfide as solvent (1% solutions, 1 mm. cell, 1 mm. compensating cell containing carbon disulfide). For measurements in the region 6.1–7 μ, where carbon disulfide absorbs strongly, tetrachloroethylene was used as solvent. No compensating cell was available at the time the α-vitamin A aldehyde curve was made which left a few gaps (curve 2).

To increase the accuracy for the data given in Table II, measurements were made both with the double beam instrument and with a Perkin–Elmer single beam instrument (Model 12A) having a calcium fluoride prism. In both cases, tetrachloroethylene was used as solvent. The wave lengths reported were determined with the single beam instrument and are considered accurate to 0.005 μ. The extinction coefficients in Table II were determined in the double beam instrument, with 1% solutions, in a 0.009″ (0.23 mm.) cell.

The data in Tables III and IV were obtained in the double beam instrument with 0.3–0.5% solutions in carbon disulfide, in a 1 mm. cell. The 10.36 μ wave length was checked with elaidic acid. Wave length measurements determined with the double beam instrument are considered accurate to 0.01 μ.

All curves are photocopies of the actual recordings except between 6.1 and 7 μ where the curves were traced.

Antimony Trichloride Reaction.—Aliquots of the aldehydes in chloroform (1 ml.), concentration approximately 0.0014%, were pipetted into matched 1 cm. glass cells and these were placed in a Hardy recording spectrophotometer. A saturated chloroform solution of antimony trichloride was immediately added (10 ml.) by means of an automatic pipet. The transmittance at 664 mμ was observed at full color development, which was 30 seconds from the addition of the antimony trichloride reagent.

Isomerization. (a) **With Pyridine Hydrochloride.**—Each of the vitamin A aldehyde isomers (*e.g.*, 0.9 g.) in methyl ethyl ketone (9 ml.) containing α-tocopherol (10 mg.) as an antioxidant, was treated with 1.5 ml. of a solution of pyridine in methyl ethyl ketone (0.81 g./100 ml.) and 3.0 ml. of a solution of hydrochloric acid in methyl ethyl ketone (0.17 g./100 ml., acid added as 35% commercial, aqueous acid). A red flask was used to exclude light. The resulting solution was refluxed for varying times (0.5 hr. for all-*trans* and neovitamin A aldehydes, 1.5 hr. for the 6-*cis* and 2,6-di-*cis* isomers, up to 2.5 hr. for α-vitamin A aldehyde). The solutions were then cooled, diluted with distilled water and extracted with ether. The ether extract was washed successively with 5% sulfuric acid, 5% potassium carbonate and water and then dried over anhydrous sodium sulfate. The solvent was removed in a nitrogen atmosphere to yield the mixed aldehydes. The isomer composition was determined, quantitatively or qualitatively, depending on the complexity of the mixture, by infrared assays.

The bands used in the infrared assays were as follows: the all-*trans* isomer has distinctive bands at 3.65, 3.71, 7.52, 8.61, 8.84, 11.43, 12.20 and 12.93 μ, those underlined being used principally. The other isomers have distinctive bands as follows: 2-cis: 9.47, 10.48, 11.91 and 13.22 μ;

6-cis: 8.73, 9.74, 11.36 μ; 2,6-di-*cis*: 14.02 μ; neoretinene-*b*: 8.93, 9.26 μ.

(b) **With Iodine.**—The aldehyde (*e.g.*, 1.0 g.) in benzene (500 ml.) containing iodine (2 mg.) was allowed to stand at room temperature under the specified conditions of time and amount of light. The work-up procedure has already been described.[23] Isomerizations run "in darkness" were done in red glassware, covered with a black cloth, and worked up in red glassware.

Reaction of Neovitamin Ab (2,4-Di-*cis*-vitamin A with Maleic Anhydride.—The procedure was the same as that used in the analytical method for neovitamin A.[23] For one sample of neovitamin Ab concentrate (*E*(1%, 1 cm.)(322 mμ) 921; the 5-cc. aliquot contained 0.35 mg. of concentrate) the percentage recovery of vitamin A, as measured by the antimony trichloride method, was 60% after reaction with maleic anhydride for 15 hours. The reaction thus proceeded slowly (apparent "neovitamin A" content = 71%), indicating the presence of a *cis* bond in the 2- or 4-position. For another sample of neovitamin Ab concentrate (*E*(1%, 1 cm.)(320 mμ) 1080) the percentage recovery of vitamin A after 15 hours was 65% (apparent "neovitamin A" content = 77.5%).

(23) C. D. Robeson and J. G. Baxter, THIS JOURNAL, **69**, 136 (1947).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF DISTILLATION PRODUCTS INDUSTRIES]

Chemistry of Vitamin A. XXVI. The Condensation of Aldehydes with Methyl β-Methylglutaconate[1]

BY JOHN D. CAWLEY

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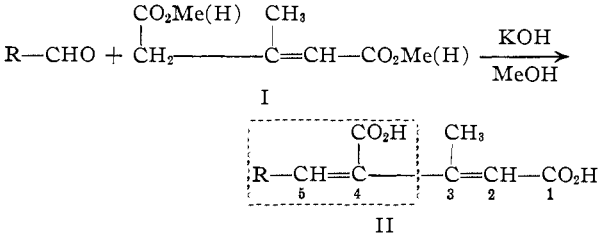
The condensation of a variety of aldehydes with methyl β-methylglutaconate to yield γ-alkylidene-β-methylglutaconic acids, R—CH=C(CO$_2$H)—C(CH$_3$)=CH—CO$_2$H, is described. The ultraviolet absorption spectra of these are consistent only with their having a *cis* relationship of the R and C(CH$_3$)=CH—CO$_2$H groups about the newly created double bond. This is also true for the 4-carboxyvitamin A acids previously prepared in this Laboratory from *trans*- and *cis*-β-ionylideneacetaldehyde and methyl β-methylglutaconate. Certain of the compounds differ in chemical and physical properties from those previously reported by Petrow and Stephenson.

In 1941 Hurd and Abernethy[2] demonstrated again[3] the ability of an ester of β-methylglutaconic acid (I) to condense with benzaldehyde to yield γ-benzylidene-β-methylglutaconic acid (II, R = C_6H_5), and suggested that this reaction with appropriate aldehydes might be used in a synthesis of vitamin A and other isoprenoid compounds.



The realization of this suggestion requires the removal of the 4-carboxyl group from II, and although Petrow and Stephenson[4] later successfully applied the reaction to other aldehydes, the diacids II obtained by them resisted all attempts at decarboxylation.

The synthesis in this Laboratory of vitamin A by the application of this reaction to β-ionylideneacetaldehyde has been described.[5] The present paper deals with the synthesis and properties of diacids II from aldehydes other than β-ionylideneacetaldehyde, but considers the geometry of the diacids obtained from this latter together with that of the other compounds.

The methylene group of β-methylglutaconic acid is less reactive than the methylene group of malonic acid, of which it is a (substituted) vinylog. In agreement with Petrow and Stephenson, it was found that the condensation of the acid or its ester with aldehydes is not mediated by amines, even under forcing conditions; a strong base is required, and nothing superior to alcoholic potassium hydroxide, as originally used by Feist and Beyer,[3] has been found. The acid itself fails to react even with this—the ester must be used.[6]

Petrow and Stephenson have reported that the reaction is applicable to alkyl, aryl and heterocyclic aldehydes, and our results also demonstrate the broad scope of the reaction. However, alkyl- (but not acyl-)aminobenzaldehydes give diacids which spontaneously decarboxylate to monoacids,[7]

(1) Communication No. 198 from this Laboratory. Presented in part before the Division of Biological Chemistry of the 126th Meeting of the American Chemical Society, New York, New York, September, 1954.

(2) C. D. Hurd and J. L. Abernethy, THIS JOURNAL, **63**, 976 (1941).

(3) F. Feist and O. Beyer, *Ann.*, **345**, 117 (1906).

(4) V. Petrow and O. Stephenson, *J. Chem. Soc.*, 1310 (1950).

(5) C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger and A. J. Chechak, THIS JOURNAL, **77**, 4111 (1955).

(6) This is reminiscent of the Stobbe condensation of carbonyl compounds with a succinic ester, but not with the acid, to yield alkylidenesuccinic half-esters (*cf.* W. S. Johnson and G. H. Daub in "Organic Reactions," Vol. VI, Ch. 1). Some evidence, based largely on equivalent weight by titration, has been found that half-esters of II may also be formed if minimal amounts of potassium hydroxide at room temperature are used. No pure compounds were isolated and the position of the ester group was not determined.

(7) J. D. Cawley and D. R. Nelan, THIS JOURNAL, **77**, 4130 (1955).