[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF DISTILLATION PRODUCTS INDUSTRIES, DIVISION OF EASTMAN KODAK COMPANY]

Chemistry of Vitamin A. XXIV. The Synthesis of Geometric Isomers of Vitamin A *via* Methyl β-Methylglutaconate[1]

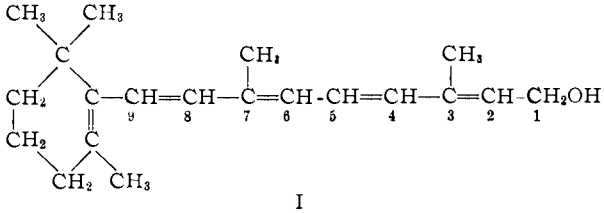
BY C. D. ROBESON, J. D. CAWLEY, L. WEISLER, M. H. STERN, C. C. EDDINGER AND A. J. CHECHAK

RECEIVED AUGUST 24, 1954

A method is described for the synthesis in pure crystalline form of four geometric isomers of vitamin A from the *cis*- and *trans*-β-ionylideneacetaldehydes *via* the intermediate 4-carboxyvitamin A acids. On the basis of certain described properties of these isomers, including their ultraviolet and infrared absorption spectra, reaction with maleic anhydride and interconversion by catalytic isomerization, assignments of their stereochemical configurations have been made. Three isomers, the all-*trans*, the 2-*cis* and the 6-*cis* appear to be identical with those already reported, while the fourth, the 2,6-di-*cis*, has not been described previously. The preparation and properties of "α-vitamin A," the structural isomer in which the ring double bond has the same position as in α-ionone, are also described.

A synthesis of vitamin A has been devised in these Laboratories which basically consists in the condensation of β-ionylideneacetaldehyde with an ester of β-methylglutaconic acid, under alkaline conditions, to form 4-carboxyvitamin A acid. The latter is decarboxylated to vitamin A acid and this, by reduction of its ester, is converted to vitamin A.[2] By separately using *trans*- and *cis*-β-ionylideneacetaldehyde in the synthesis, four geometrical isomers of vitamin A have been isolated in pure crystalline form.[3]

The studies indicated that these isomers have the configurations: all-*trans*, 2-*cis*, 2,6-di-*cis* and 6-*cis*, according to the following numbering system[4]



I

The configuration at the double bonds not mentioned is understood to be *trans*-.

The synthetic all-*trans*- and 2-*cis*-vitamin A isomers correspond in assigned configuration and properties to those which occur naturally in fish liver oils.

The 6-*cis*-vitamin A isomer corresponds in assigned configuration and melting point with the *cis*-(6-*cis*)-vitamin A of Graham, *et al.*,[5] prepared by a different method.

These compounds thus represent the four possible unhindered isomers based on the early concept of the "stereochemically effective" double bonds in vitamin A and other carotenoids.[6] A fifth vitamin A isomer described in another publication[7] probably contains a sterically hindered *cis*-double bond in the 4-position since it was prepared from neoretinene-b[8,7] for which a 2,4-di-*cis* configuration has been proposed on the basis of infrared studies.[9] Other examples of the synthesis of isoprenic polyenes having sterically hindered *cis*-double bonds have been described[10,11] and the general subject of polyene stereoisomerism has been recently reviewed.[12]

Synthesis of Intermediates

1. *cis*- and *trans*-Methyl β-Ionylideneacetates (II).—β-Ionone was converted to the individual *cis*- and *trans*-methyl β-ionylideneacetates (isomerism about double bond starred, formula II), *via* the corresponding β-ionylideneacetic acids. This served to fix the configuration at carbon atom 6 of vitamin A (I), in *cis*- and *trans*-positions. The individual *cis* and *trans* isomers of the acids were obtained by fractional crystallization of the mixed acids prepared by saponifying "ethyl β-ionylideneacetate."[13]

β-Ionylideneacetic acid (m.p. 126–127°) obtained by fractional crystallization, has been fre-

(1) Communication No. 205. 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) The synthetic method was also applied to α-ionylideneacetaldehyde to prepare an analog of vitamin A which we shall call α-vitamin A. No attempt to isolate individual isomers was made, however (see Experimental Part). This work was done to compare the ultraviolet absorption properties of the intermediates in the two series to detect whether migration of the ring double bond occurred during the synthesis of the vitamin A isomers. There was no evidence of this.

(3) The effect of configuration on the biological activity of the isomers, as determined by rat, liver-storage and growth tests, is described in a separate communication from these laboratories (ref. 32).

(4) The numbering system used in this paper corresponds to that in "Chemical Abstracts, 1953 Subject Index."

(5) W. Graham, D. A. van Dorp and J. F. Arens, *Rec. trav. chim. Pays-Bas*, **68**, 609 (1949). These authors offered no proof of a 6-*cis* configuration for the isomer which they prepared other than its synthesis from *cis*-C_{18} ketone.

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

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

(8) R. Hubbard, R. Gregerman and G. Wald, *J. Gen. Physiol.*, **36**, 415 (1953).

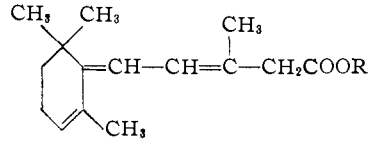
(9) C. D. Robeson, W. P. Blum, J. M. Dieterle, J. D Cawley and J. G. Baxter, THIS JOURNAL, **77**, 4120 (1955).

(10) W. Oroshnik, G. Karmas and A. D. Mebane, *ibid.*, **74**, 295 (1952).

(11) C. F. Garbers, C. H. Eugster and P. Karrer, *Helv. Chim. Acta*, **35**, 1850 (1952); **36**, 562 (1953); **36**, 828 (1953).

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

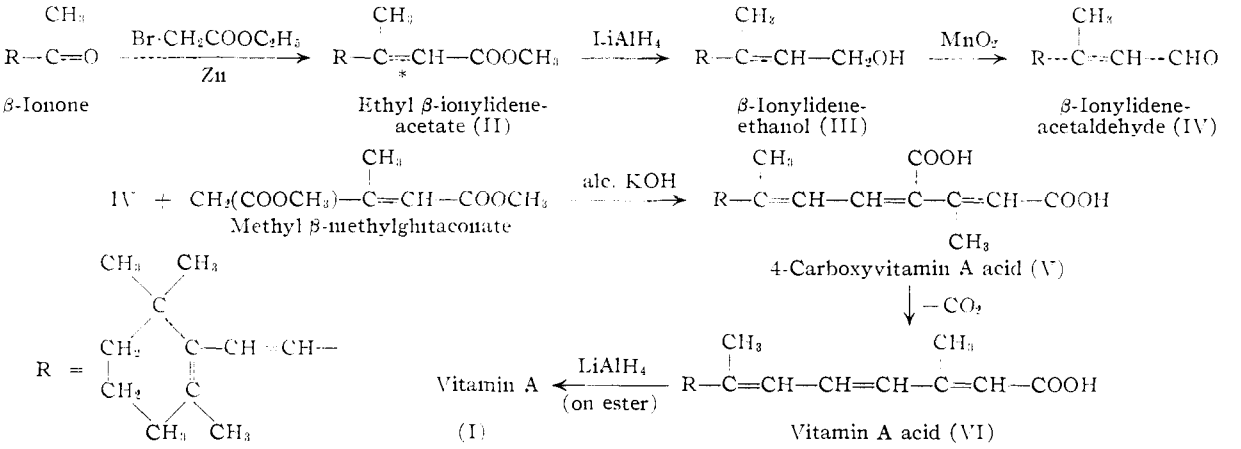
(13) P. Karrer, H. Salomon, R. Morf and O. Walker, *Helv. Chim. Acta*, **15**, 878 (1932). The ester prepared by this method contains in addition to the *cis*- and *trans*-β-ionylideneacetates, a third isomer



in which the carbon-carbon double bond system is not conjugated with the ester carbonyl. The structure of this ester and its next higher isoprenolog has been described earlier: (a) W. Oroshnik, G. Karmas and A. D. Mebane, THIS JOURNAL, **74**, 3807 (1952); (b) H. O. Huisman, A. Smith, S. Vromen and L. G. M. Fisscher, *Rec. trav. chim. Pays Bas*, **71**, 899 (1952); (c) J. G. Baxter, *Fortschr. Chem. organ. Naturstoffe*, **9**, 41 (1952). This type of configuration containing an exocyclic double bond has been termed "retroionylidene" by Oroshnik, *et al.*, ref. 10.

quently described[13-15] and characterized as the *trans* isomer.[14] The lower melting *cis* isomer (m.p. 98.5–99.5°), which we prepared by further fractional crystallization, has not been previously reported. Its ultraviolet absorption curve (Fig. 1) is similar in shape to that of the *trans* isomer and although the main maximum lies at a longer wave length (306 mμ) than that of the *trans* compound (297 mμ), it has the expected lower extinction coefficient.

The methods of synthesis for the individual isomers are summarized in Scheme I.



SCHEME I

Both the methyl and ethyl esters of the two β-ionylideneacetic acids were prepared by esterification under non-isomerizing conditions (see Experimental Part) but only the *trans*-methyl ester (m.p. 17.5°) was obtained in crystalline form. Conversion of the acids to their esters caused a shift of approximately 6 mμ in the ultraviolet absorption maxima to a longer wave length.

2. ***cis*- and *trans*-β-Ionylidene-ethanols (III).**—These were prepared by reduction of *cis*- and *trans*-methyl β-ionylideneacetates with lithium aluminum hydride as earlier described for the mixed esters.[16] The anomalous ultraviolet absorption spectra of certain reported preparations[15,17] of β-ionylidene-ethanol was apparently due to the presence of the "retro" isomer since they were prepared from the corresponding mixed ethyl β-ionylideneacetates.[13]

The *cis* isomer of β-ionylidene-ethanol has not been previously reported. Of particular interest with respect to ultraviolet absorption spectra (Fig. 2) is the fact that the main maximum for the *cis* isomer (266 mμ) is at a longer wave length than that for the *trans* isomer (259 mμ) although the *cis* compound has the expected lower extinction coefficient. Thus, this property of *cis*-β-ionylidene-ethanol is consistent with the earlier observation[18] that neovitamin A, in which the terminal double bond is also *cis*-, absorbs at a longer wave length than the all-*trans* isomer.

3. ***cis*- and *trans*-β-Ionylideneacetaldehydes (IV).**—These were prepared by oxidizing *cis*- and *trans*-β-ionylidene-ethanols with manganese dioxide according to a method earlier described for oxidizing vitamin A to vitamin A aldehyde.[19]

The absorption maxima (Fig. 1) of the aldehyde isomers lie in a reverse relative position compared with the corresponding *cis*- and *trans*-β-ionylideneacetic acids and β-ionylidene-ethanols, the maximum of the *trans*-aldehyde occurring at longer wave length than that of the *cis* isomer. The expected higher extinction coefficient for the *trans* isomer was observed.

Wendler, *et al.*,[15] have described a *cis*-β-ionylideneacetaldehyde whose ultraviolet absorption properties differ from ours,[20] although the melting point and ultraviolet absorption properties reported for the two semicarbazone derivatives are in good agreement. The Wendler preparation was obtained by chromatographic separation of the mixed aldehyde isomers. We have also prepared the *cis* isomer by a similar chromatographic procedure but

(11) W. G. Young, L. J. Andrews and S. J. Cristol, THIS JOURNAL, **66**, 520 (1944).

(15) N. L. Wendler, H. L. Slates, N. R. Trenner and M. Tishler, *ibid.*, **73**, 719 (1951).

(16) J. H. Cawley, C. D. Robeson, E. M. Shantz, L. Weisler and J. G. Baxter, U. S. Patent 2,576,193 (1951).

(17) N. A. Milas and T. M. Harrington, THIS JOURNAL, **69**, 2247 (1947).

(18) C. D. Robeson and J. G. Baxter, *ibid.*, **69**, 136 (1947).

(19) S. Ball, T. W. Goodwin and R. A. Morton, *Biochem. J.*, **42**, 516 (1948).

(20) Wendler, *et al.*, reported E (1%, 1 cm.)(318 mμ) = 904 (isooctane) for the compound. We found E(1%, 1 cm.)(258, 305 mμ) = 448, 518 (isooctane).

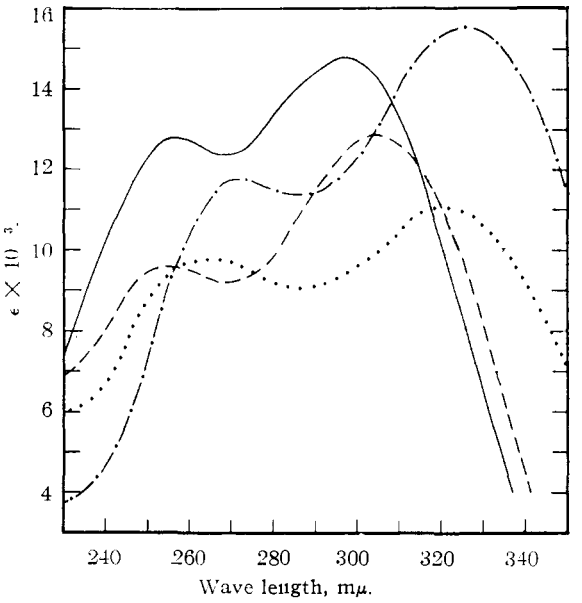


Fig. 1.—Ultraviolet absorption spectra of: *trans*-β-ionylideneacetic acid (—); *cis*-β-ionylideneacetic acid (------); *trans*-β-ionylideneacetaldehyde (·····); *cis*-β-ionylideneacetaldehyde (. . . . .).

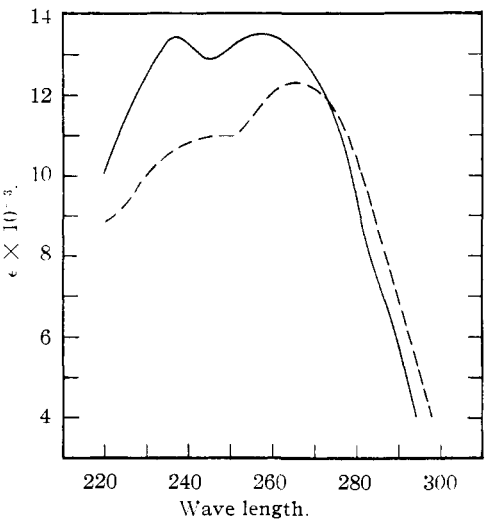


Fig. 2.—Ultraviolet absorption spectra of: *trans*-β-ionylidene-ethanol (—); *cis*-β-ionylideneëthanol (------).

found it to have the same ultraviolet absorption properties as the compound made by oxidation of pure *cis*-β-ionylidene-ethanol. This anomaly deserves further study.

The properties of our *trans*-β-ionylideneacetaldehyde agreed well with those reported by Wendler, *et al.*,[15] and Huisman, *et al.*[13b] Other preparations of β-ionylideneacetaldehyde,[21-23] made by different methods, appear to have contained a substantial amount, at least, of the *trans* form, judging from the reported properties for their derivatives.

4. C- and D-4-Carboxyvitamin A Acids (V).—When *trans*-β-ionylideneacetaldehyde was condensed with methyl β-methylglutaconate under strongly alkaline conditions,[24] there resulted almost exclusively and in excellent yield a 4-carboxyvitamin A acid which for convenience we have called C-diacid. For the diacid prepared in similarly good yield from *cis*-β-ionylideneacetaldehyde, we have used the prefix D-. Evidence is presented in another paper from these Laboratories[25] to support the conclusion that the double bond created at carbon atom 4 in these diacids has a *cis* configuration.

Petrow and Stephenson[26] have also investigated the condensation of β-ionylideneacetaldehyde, made by another procedure, with an ester of β-methylglutaconic acid. Surprisingly, neither our C- nor D-diacids conform in melting point and ultraviolet absorption properties with the 4-carboxyvitamin A acids they have described.

(21) R. Kuhn and C. J. O. R. Morris, *Ber.*, **70**, 853 (1937).

(22) C. D. Robeson and C. C. Eddinger, U. S. Patent 2,507,647 (1950).

(23) J. F. Arens, D. A. van Dorp, G. van Dijk and B. J. Brandt, *Rec. trav. chim. Pays Bas*, **67**, 973 (1948).

(24) The condensation of aldehydes with β-methylglutaconic esters was first described by F. Feist and O. Beyer, *Ann.*, **345**, 117 (1906).

(25) J. D. Cawley, THIS JOURNAL, **77**, 4125 (1955).

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

Similar discrepancies between the properties of diacids prepared from other aldehydes in the two laboratories are discussed by Cawley.[25] The difference, at least in the case of the 4-carboxyvitamin A acids, appears to be stereochemical since the anhydride which Petrow and Stephenson prepared from their 4-carboxyvitamin A diacids by heating with acetic anhydride appears to be identical with that which we obtain from either the C- or D-diacids under similar conditions.

Our C- and D-diacids differed markedly in the solubilities of their potassium salts in ethyl alcohol. The salt of C-diacid was nearly insoluble while that of D-diacid was readily soluble. In this respect, the Petrow–Stephenson "*trans*-4-carboxyvitamin A acid" appeared similar to our C-diacid since they obtained their diacid from the potassium salt which was insoluble in alcohol.

Petrow and Stephenson reported that their 4-carboxyvitamin A acids could not be decarboxylated to form vitamin A acid. Our diacids, however, were readily decarboxylated to vitamin A acids when heated in solution in an organic base (*e.g.*, pyridine or 2,4-lutidine), containing small amounts of copper powder or copper salts, such as the acetate. In each case, about a 65% yield of vitamin A acid isomer was obtained.

Neo- and All-*trans*-vitamin A

Decarboxylation, as described above, of C-vitamin A diacid gave almost exclusively 2-*cis*- or neovitamin A acid[27] which crystallized readily from methyl alcohol in the form of reddish-orange plates having a melting point of 174–175° and an absorption maximum at 354 mμ (ε 39,800). A photomicrograph of the crystals has previously been published.[13c]

Evidence that this monoacid corresponds in

(27) A vitamin A acid with quite different properties (m.p. 146°, ε(347 mμ) 48,000) has been described and assigned a 2-*cis*- structure (H. H. Inhoffen, F. Bohlmann and M. Bohlmann, *Ann.*, **568**, 47 (1950)). We could not obtain this acid by the method which these authors described and have never found it, in spite of careful searches, among the vitamin A acid isomers we have prepared by other procedures. The reason for this needs clarification.

TABLE I

PROPERTIES OF VITAMIN A ISOMERS[a]

Vitamin A isomer	M.p., °C.	λ_{max}, mμ	ε	*p*-Phenylazobenzoate ester M.p., °C.	Relative reaction rate with maleic anhydride
Neovitamin A (natural)	58–60	328	47,000	94-96	Slow
Neo- or 2-*cis*-vitamin A (from neovitamin A acid)	58–59	328	48,300	94–95	Slow
All-*trans*-vitamin A (natural)	62–64	325	51,000	79–80	Fast
All-*trans*-vitamin A (from *trans*-vitamin A acid)	64	325	52,800	79-80	Fast
2,6-Di-*cis*-vitamin A	58–59	324	39,500	91.5-92.5	Slow
6-*cis*-Vitamin A	81.5-82.5	323	42,300	79-80	Fast

[a] Ultraviolet absorption measurements in ethanol.

configuration to neovitamin A was obtained when its methyl ester was reduced with lithium aluminum hydride, at low temperatures (−50°), to a vitamin A isomer which had identical properties (Table I) with those of the natural neovitamin A.[18] Partial conversion to all-*trans*-vitamin A occurred during reduction of the ester if the reaction time was extended or if a low temperature was not maintained, and the reaction product then contained all-*trans*-vitamin A as well as neovitamin A. Whether this conversion arose from isomerization of the neovitamin A acid methyl ester or of neovitamin A is not known.

The conversion of neovitamin A acid to the all-*trans* acid (m.p. 180°, ε(350 mμ) 45,200) was accomplished by isomerization with iodine (Experimental Part). The *trans*-acid was similar in properties to the all-*trans* vitamin A acid first prepared by van Dorp and Arens.[28]

All-*trans*-vitamin A with the properties of the natural vitamin (Table I) was prepared by the reduction of the methyl ester of the *trans*-acid, with lithium aluminohydride.

All-*trans*-vitamin A acetate also was prepared by isomerization of neovitamin A acetate by a method earlier used to convert the anthraquinone carboxylate ester of natural neovitamin A to the corresponding all-*trans* ester.[18] The ratio of vitamin A acetate to neovitamin A acetate in the equilibrium mixture was 68:32 as determined by the maleic anhydride assay method[18] and the crystalline acetate isolated from the mixture had the same melting point and ultraviolet absorption properties as natural all-*trans*-vitamin A acetate.[29]

This completed the cycle of transformations for the *trans* series shown in Scheme I and supported the earlier tentative assignment[18] of a 2-*cis*,6-*trans* configuration for neovitamin A.

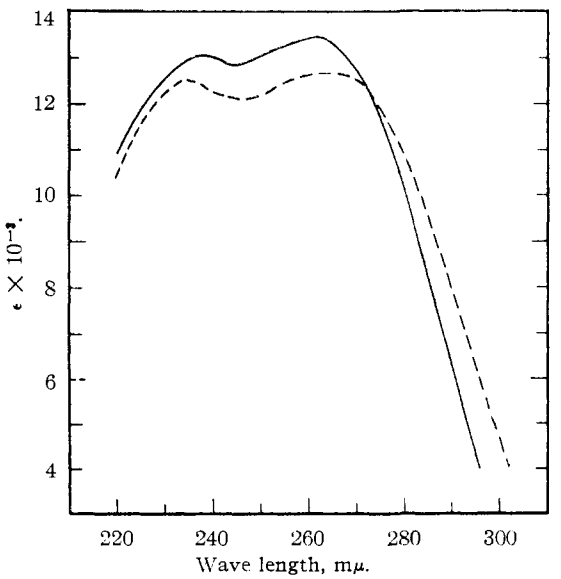


Fig. 3.—Ultraviolet absorption spectra of maleic anhydride adducts of: all-*trans*-vitamin A acetate (—), 6-*cis*-vitamin A acetate (------).

Reaction with Maleic Anhydride.—Further support for the 2-*cis*,6-*trans* configuration for neovitamin A was provided by more extensive studies of the reaction of all-*trans*-vitamin A with maleic anhydride.

In these studies it was demonstrated that the two terminal double bonds in the 2- and 4-positions are the only ones involved when reaction occurs. The shape of the ultraviolet absorption curve (Fig. 3) and the position of the maximum (261 mμ) for the adduct isolated from the reaction of the acetate ester of the all-*trans* isomer was essentially the same as for *trans*-β-ionylidene-ethanol. This would be expected if only the two terminal bonds of the conjugated double bond system participated.[30] Thus a *cis* configuration at the terminal 2-position of neovitamin A explains the lack of reactivity of this isomer with maleic anhydride.

It was also evident from the ultraviolet absorption curve of the maleic anhydride adduct of all-*trans*-vitamin A acetate that there was no stereochemical change in the triene system originally present in *trans*-β-ionylidene-ethanol during the succeeding steps of the synthesis, thus confirming the 6-*trans* configuration for both neovitamin A and all-*trans*-vitamin A.

Ultraviolet Absorption Spectra.—The ultraviolet absorption curves for the synthetic all-*trans*- and neovitamin A (Fig. 4) were similar to those previously given[18] for the natural vitamin except that the extinction coefficients at the maxima (325, 328 mμ, respectively) were slightly higher.

The ultraviolet absorption spectrum of neovitamin A, which at one time appeared anomalous because the position of the maximum was at a longer wave length than that of the all-*trans*-

(28) D. A. van Dorp and J. F. Arens, *Rec. trav. chim.*, **65**, 338 (1946).

(29) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2407 (1942).

(30) The demonstrated lack of reactivity of the triene system toward maleic anhydride was further tested by treating the acetate of *trans*-β-ionylidene-ethanol with the reagent. There was no reaction under the conditions of the experiment.

vitamin A has been shown to be in harmony with the similar bathochromic effect observed for *cis*-β-ionylidene-ethanol, a compound in which the *cis*-double bond is also allylic to the alcohol group.

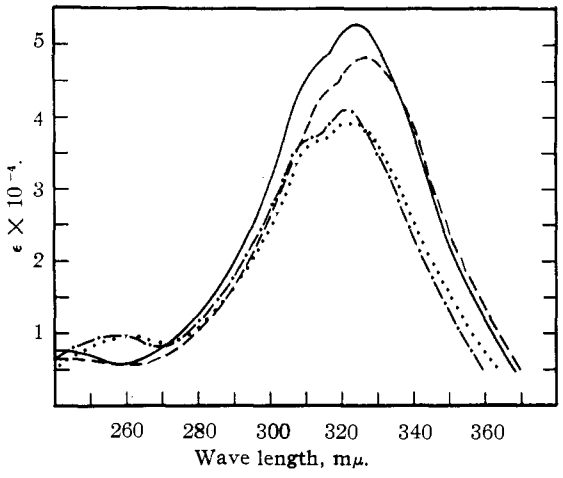


Fig. 4.—Ultraviolet absorption spectra of: all-*trans*-vitamin A (—); 2-*cis*-vitamin A (------); 6-*cis*-vitamin A (·-·-·-·); 2,6-di-*cis*-vitamin A (. . . . .).

Infrared Absorption Spectra.—The infrared absorption curves[31] of all-*trans*-vitamin A and neo- or 2-*cis*-vitamin A are shown in Fig. 5 (curves 1 and 2, respectively). These curves are identical over a considerable portion of the spectrum but differ with respect to the positions and character of certain of the absorption bands.

In the region near 10 μ at which the absorption due to the C–O stretching vibration might reasonably be expected to be influenced by the configuration of the nearest (terminal) double bond, the all-*trans* isomer has a maximum at 10.05 μ while the 2-*cis* isomer has its maximum at 9.98 μ.

Other differences are evident in the stronger absorption of a band at 9.3 mμ for the all-*trans* isomer than of a corresponding band at 9.2 mμ for the 2-*cis* compound and in the position of a band which occurs at 12.25 mμ for the *trans*- and at 12.0 μ for the 2-*cis* isomer.

2,6-Di-*cis*- and 6-*cis*-Vitamin A

Decarboxylation of D-vitamin A diacid gave almost exclusively a vitamin A acid having a lower melting point (135–136°) than any vitamin A acid previously described. The maximum of its ultraviolet absorption curve was at 346 mμ (ε 34,500), a wave length shorter by 4 mμ than that for the maximum of all-*trans*-vitamin A acid.

By isomerization of this acid with iodine, an isomerate was obtained from which another acid was obtained by crystallization (m.p. 189–190°, ε(345 mμ) 36,900).

The conversion of these two new acids to the all-*trans* isomer could not be accomplished by isomerization with iodine. However, treatment of the methyl ester of either one with hydrochloric acid in acetonitrile solution gave isomerates from which the all-*trans*-vitamin A acid could be isolated, after saponification. Since under these conditions, the isomerate contained some of the retroionylidene isomer, it appears that this isomer may be an intermediate, the shifting of the double bonds preceding the stereochemical change.

(31) The infrared absorption spectra were determined and interpreted by Mr. W. Blum and assistants of these Laboratories. The measurements were made on 1.5% solutions of the isomers in carbon disulfide solution in a 1-mm. cell, using a Perkin Elmer recording infrared spectrophotometer, Model 21, equipped with a sodium chloride prism. The portion of the curves from 6 to 7.2 μ was determined in tetrachloroethylene.

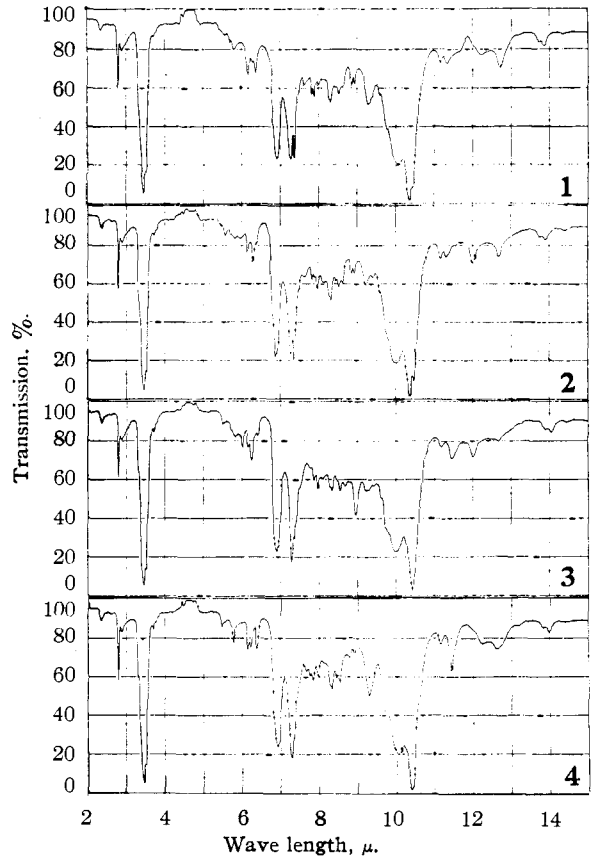


Fig. 5.—Infrared absorption spectra of: (1) all-*trans*-vitamin A; (2) 2-*cis*-vitamin A, (3) 2,6-di-*cis*-vitamin A; (4) 6-*cis*-vitamin A.

By analogy with the preparation of neovitamin A acid and all-*trans*-vitamin A acid from *trans*-β-ionylideneacetaldehyde, it seemed reasonable to assume the 2,6-di-*cis* and 6-*cis* configurations, respectively, for this pair of acids from the corresponding *cis* intermediate. The correctness of this assumption was supported by subsequent studies on the vitamin A isomers prepared from them.

The vitamin A isomers obtained by reduction with lithium aluminohydride of the methyl esters of this new pair of vitamin A acids were purified by crystallization from ethyl formate. Properties of these (the 2,6-di-*cis* and the 6-*cis* isomers) and of their *p*-phenylazobenzoate esters are shown in Table I.

The *p*-phenylazobenzoate of the 6-*cis* isomer was also prepared by isomerization of the 2,6-di-*cis* ester with iodine. A mixture of isomers was obtained which analyzed by the maleic anhydride method for 68.5% of a 2-*trans* isomer. By crystal-

lization, the *p*-phenylazobenzoate of the 6-*cis* compound was separated, thus completing the transformations for the *cis* series (Scheme I).

Reaction with Maleic Anhydride.—Confirmation of the 2-*cis* configuration for the vitamin A from the reduction of the methyl ester of the acid melting at 135–136° and the 2-*trans* configuration for that from the acid melting at 189–191° was obtained from a study of the reaction of their *p*-phenylazobenzoate esters with maleic anhydride (Fig. 6). The difference in rate of this reaction for the two isomers (curves D and F) was exactly analogous to the difference in rate for the corresponding esters of neovitamin A and all-*trans*-vitamin A (curves C and B).

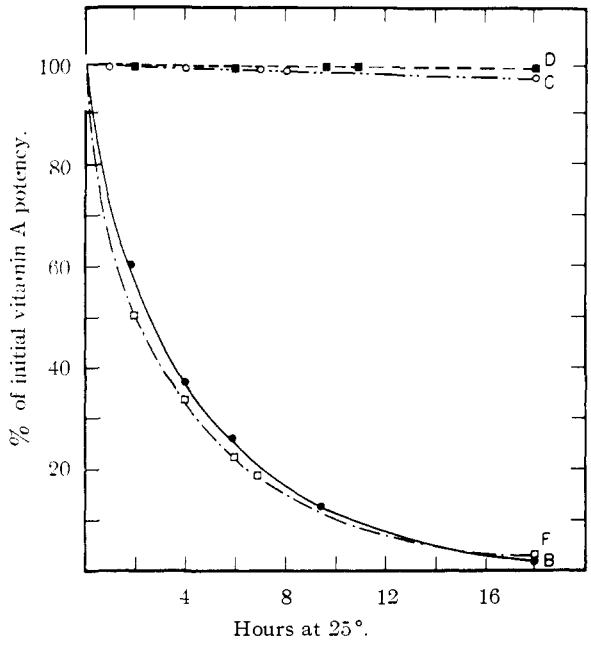


Fig. 6.—Reaction of *p*-phenylazobenzoate esters of vitamin A isomers with maleic anhydride: curve B, all-*trans*-vitamin A; curve C, 2-*cis*-vitamin A; curve D, 2,6-di-*cis*-vitamin A; curve F, 6-*cis*-vitamin A.

Further evidence for the stereospecificity of the synthesis and hence for the 6-*cis* configuration for the isomers derived from *cis*-β-ionylidene-ethanol was apparent from the ultraviolet absorption spectrum of the maleic anhydride adduct of the acetate of the 2-*trans* isomer in this series (Fig. 3). The position of the maximum (265 mμ) and molecular extinction coefficient for the adduct conformed with those for the same triene system in *cis*-β-ionylidene-ethanol.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra of the 6-*cis* isomers are compared with those of the two 6-*trans* isomers in Fig. 4. The position of the maximum for the 2,6-di-*cis*-vitamin A (324 mμ) was at a lower wave length than that of the all-*trans* isomer and its extinction coefficient was lowest of the four. The change in wave length was, however, smaller than observed among certain carotenoids.[12]

The hypsochromic shift in absorption maximum which accompanies the conversion of the 2,6-di-*cis* to the 6-*cis* isomer (323 mμ) appears to be characteristic of a change from a *cis*- to a *trans*-configuration at the terminal double bond and is in accord with the similar shift for the neovitamin A–vitamin A and *cis*- and *trans*-β-ionylidene-ethanol pairs.

The 6-*cis* isomers are distinguished from the two 6-*trans* isomers in that they have subsidiary maxima at about 260 mμ.

The demonstration that 6-*cis*-vitamin A isomers can be produced by synthesis and that their ultraviolet absorption properties are different from those of the 6-*trans* isomers promises to present new problems in the physicochemical analysis of synthetic vitamin A concentrates whose freedom from undesired isomers is not insured by the method of synthesis. This is particularly true since the 6-*cis* isomers are shown, in another publication from these laboratories,[32] to be lower in biological potency than the 6-*trans* isomers.

Infrared Absorption Spectra.—The infrared absorption spectra of this pair of vitamin A isomers is shown in Fig. 5 (curves 3 and 4). These spectra appear to be consistent with the configurations assigned to the compounds on the basis of other evidence.

The position of the bands at 9.2, 9.98 and 12.0 μ for the 2,6-di-*cis* isomer coincide with those for the 2-*cis*-vitamin A while the corresponding bands at 9.3, 10.05 and 12.25 μ for the 6-*cis* isomer occur at the same wave length as those for all-*trans*-vitamin A. Furthermore, both 2-*cis* isomers show considerably weaker absorption in the 9.2–9.3 μ region than observed for the corresponding 2-*trans* compounds.

The isomers having 6-*cis* configurations are distinguished from the 6-*trans* isomers by the character of the "*trans*-peak" at 10–10.6 μ, the band due to the *trans* "unsubstituted" carbon–carbon double bonds, such as at positions 4 and 8 in the side chain of vitamin A. The maxima occur at 10.40 μ for the 6-*cis* isomers while those for the 6-*trans* isomers consist of double peaks, a main maximum at 10.35 and a subsidiary one at 10.45. Since all four isomers show equivalent extinction in this range, they must all contain the same number of *trans* —CH=CH— bonds.

The 6-*cis* isomers are further distinguished from those having a *trans*-configuration at this position by the character of the absorption in the range 7.28–7.36 μ. Peaks in this region are frequently associated with the hydrogen bending of methyl groups although Zechmeister[12] has reported a 7.25 μ band for the *cis* isomers of diphenylbutadiene as well as certain *cis*-carotenoids, not found in curves for the corresponding all-*trans* isomers. Although all four isomers have maxima at both 7.28 and 7.36 μ, only for the 6-*trans* isomers is the peak at 7.36 μ well defined and comparable in strength with that at 7.28 μ.

Certain bands were unaffected by geometric isomerism. These included such bands as the sharp main band at 2.77 μ due to the -O-H stretching vibration.

(32) S. R. Ames, W. J. Swanson and P. L. Harris, THIS JOURNAL, 77, 4134 (1955).

Experimental Part

All ultraviolet absorption spectra were determined in absolute ethanol unless otherwise noted. Melting points were determined in capillary tubes, using 3-inch immersion thermometers. The microanalyses were done by Mr. Donald Ketchum, Eastman Kodak Company Research Laboratories.

cis- and *trans*-β-**Ionylideneacetic Acids.**—A mixture containing the isomeric β-ionylideneacetic acids was obtained by saponifying "ethyl β-ionylideneacetate," prepared by the directions of Karrer, *et al.*[13] The acids (724 g., *E*(1%, 1 cm.) (284 mμ) 772) were dissolved in acetonitrile (800 cc.) and crystallized at 5° to yield *trans*-β-ionylideneacetic acid[15] (147 g.) which was further purified by recrystallization from ethyl formate: colorless prisms; m.p. 126-127°; ε (258,297 mμ) 12800, 14800.

The filtrate on standing at room temperature for several days deposited a second crop of crystals (11.6 g.) consisting of *cis*-β-ionylideneacetic acid. This was recrystallized from petroleum ether (b.p. 60–71°) at 5°: transparent, prismatic needles: m.p. 98.5–99.5°; ε (256,306 mμ) 9600, 13000.

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.9; H, 9.5. Found: C, 76.8; H, 9.1.

The *trans* acid was produced when a solution of the *cis* acid (0.4 g.) in isopropyl ether (8 cc.) containing iodine (4 mg.) was allowed to stand at room temperature for two hours. The solution was filtered through powdered sodium thiosulfate and the solvent evaporated in a stream of nitrogen. On cooling a solution of the residue in ethanol (4 cc.) to −15°, the *trans* acid (100 mg.) was obtained (m.p. 124–126°).

Esters of *cis*- and *trans*-β-Ionylideneacetic Acids.—The methyl and ethyl esters of *cis*- and *trans*-β-ionylideneacetic acids were prepared from the crystalline acids by treatment with the proper alkyl iodide in methyl ethyl ketone solution in the presence of anhydrous potassium carbonate, a procedure[33] which avoids isomerization to the retroionylidene isomer. The *trans*-methyl ester crystallized from methanol at −15°; the other esters were not further purified. The ultraviolet absorption data were

Ester	State	λmax, mμ	ε
trans-Methyl	Crystals, m.p. 17.5°	258, 302	12,900, 15,200
trans-Ethyl	Oil	257, 302	12,600, 15,300
cis-Methyl	Oil	257, 310	8,950, 12,900
cis-Ethyl	Oil	260, 310	9,900, 13,500

Ethyl α-Ionylideneacetate.—The intermediate hydroxy ester was prepared from α-ionone (32 g.) and ethyl α-bromoacetate by the Reformatsky reaction, according to the method of Karrer, *et al.*,[13] for the preparation of the corresponding ester from β-ionone. A benzene solution (200 cc.) of the hydroxy ester containing *p*-toluenesulfonic acid (2 g.) was refluxed 1 hour. After washing successively with 0.5 *N* potassium hydroxide solution and water, the solvent was removed by distillation at reduced pressure to give a concentrate of ethyl α-ionylideneacetate (37 g., *E* (1%, 1 cm.) (270 mμ) 780). There was no indication of a "retro" isomer being present.

By adsorption on sodium silico aluminate[34] the extinction coefficient was increased to *E* (1%, 1 cm.) (270 mμ) 978. This corresponds to ε 25,800, a value higher than that reported by Young, *et al.*[14] (ε(272 mμ) 14,700, 95% ethanol).

cis- and *trans*-β-**Ionylidene-ethanols.**—To a stirred solution of the methyl ester of *trans*-β-ionylideneacetic acid (30 g.) in anhydrous ethyl ether (200 cc.) was added dropwise an ether solution (120 cc., 0.75 molar) of lithium aluminum hydride. The temperature was maintained at about 20° by external cooling. Stirring was continued for an additional 5 minutes and the excess lithium aluminum hydride was destroyed by careful dropwise addition of 1 *N* aqueous sulfuric acid.

The ether solution was washed twice with 200 cc. of dilute sulfuric acid, twice with 200 cc. of 3% potassium hydroxide solution and finally with water to neutrality. After drying over anhydrous sodium sulfate, the ether was distilled, the last traces under reduced pressure, to yield *trans*-β-ionylidene-ethanol[13b] (26.2 g.), light yellow oil; ε (237,259 mμ) 13400, 13500. A similar preparation was made by reducing *trans*-β-ionylideneacetic acid.

Reduction of *cis*-β-ionylideneacetic acid (8.0 g.) with lithium aluminum hydride (50 cc., 0.75 molar), in the same way, gave *cis*-β-ionylidene-ethanol (7.5 g.), pale yellow oil; ε (240,266 mμ) 10800, 12300.

Anal. Calcd. for $C_{15}H_{24}O$: C, 81.8; H, 11.0. Found: C, 82.2; H, 11.0.

α-Ionylidene-ethanol.—Reduction of ethyl α-ionylideneacetate (18.5 g., *E* (1%, 1 cm.)(270 mμ) 780) with lithium aluminum hydride, as described for the β-ionylidene ester gave a concentrate of α-ionylideneëthanol (15.7 g., *E* (1%, 1 cm.)(240 mμ) 985).

cis- and *trans*-β-**Ionylideneacetaldehydes.**—To a solution of *trans*-β-ionylidene-ethanol (22.6 g.) in petroleum ether (110 cc., b.p. 30–60°) was added manganese dioxide powder[35] (130 g.) with care to prevent the reaction temperature from rising above 30°. The reaction mixture was allowed to stand at room temperature for 24 hours, filtered, and the manganese dioxide washed with fresh petroleum ether. After distillation of solvent from the filtrate, under reduced pressure, *trans*-β-ionylideneacetaldehyde (21.8 g.) was obtained; orange oil, ε (273,326 mμ) 11800, 15600.

Anal. Calcd. for $C_{15}H_{22}O$: C, 82.5; H, 10.1. Found: C, 82.5; H, 10.1.

The semicarbazone[15] was crystallized from ethanol, m.p. 194–195°, ε 35,500 (chloroform). The *p*-nitrobenzhydrazone was crystallized from benzene–ethanol, m.p. 210.5–211°, ε (344 mμ) 26,500. The 2,4-dinitrophenylhydrazone[36] was crystallized from ethanol–chloroform, m.p. 198°, ε (408 mμ) 37,000 (chloroform).

A solution of *cis*-β-ionylidene-ethanol (5.4 g.) in petroleum ether (36 cc., b.p. 30–60°), oxidized in the same way, gave *cis*-β-ionylideneacetaldehyde (yellow oil, 5.1 g., ε (266,321 mμ) 9750, 11100.

Anal. Calcd. for $C_{15}H_{22}O$: C, 82.5; H, 10.1. Found: C, 82.4; H, 10.5.

The semicarbazone[15] was crystallized from ethanol, m.p. 172–174°, ε (316 mμ) 29,700 (chloroform). The 2,4-dinitrophenylhydrazone was crystallized from ethanol–chloroform, m.p. 193°, ε (400 mμ) 33,800 (chloroform).

A sample of *cis*-β-ionylideneacetaldehyde also was prepared by chromatography of a mixture of *cis*- and *trans*-β-ionylideneacetaldehydes on sodium silicoaluminate.[34] The *cis* isomer appeared in the least strongly adsorbed fraction; ε (267,321 mμ) 9800, 11300.

α-Ionylideneacetaldehyde.—By oxidation of α-ionylideneethanol (15.5 g.) with manganese dioxide, α-ionylideneacetaldehyde was obtained (15 g., *E* (1%, 1 cm.)(285 mμ) 860); 2,4-dinitrophenylhydrazone, m.p. 174–176°; *E* (1%, 1 cm.)(396 mμ) 868.

Anal. Calcd. for $C_{21}H_{26}O_4N_4$: C, 63.3; H, 6.6; N, 14.1. Found: C, 63.3; H, 6.5; N, 14.0.

4-Carboxyvitamin A Acids. C-Diacid.—To a solution of *trans*-β-ionylideneacetaldehyde (21 g., 0.096 mole) and methyl β-methylglutaconate[37] (17.2 g., 0.10 mole) in methyl alcohol (60 cc.) was added a solution of potassium hydroxide (17 g.) in methanol (105 cc.). The reaction mixture was allowed to stand for 64 hours at 25°. Additional potassium hydroxide (30 g. in 210 cc. water) was then added and the solution refluxed (1/2 hour) to complete the saponification to the dicarboxylic acid. After addition of water (100 cc.) the alkaline solution was extracted twice with ether (200 cc. each) to remove unreacted aldehyde and other non-acidic impurities and then acidified by the addition of 10% sulfuric acid (500 cc.). After three extractions with ethyl ether (500 cc. each) of the acidified solution, the extracts were combined, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed by distillation under reduced pressure to yield a concentrate of C-diacid (yellow solid, 29 g., *E* (1%, 1 cm.)(334 mμ) 753).

To a stirred solution of the yellow solid in ethanol (220

(33) C. D. Robeson, U. S. Patent 2,583,594 (1952).

(34) "Doucil," Philadelphia Quartz Company.

(35) "Precipitated" grade, General Metallic Oxides Company.

(36) I. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 1823 (1949).

(37) This ester was prepared by base-catalyzed methanolysis of ethyl isodehydroacetate (F. R. Goss, C. K. Ingold and J. F. Thorpe, *J. Chem. Soc.*, **123**, 327 (1923)) as described for the ethyl ester (N. Bland and J. F. Thorpe, *ibid.*, **101**, 1557 (1912)). See also reference 25.

cc.) was added 10% alcoholic potassium hydroxide (900 cc.). After 1.5 hours at 25°, the precipitated potassium salt was filtered and washed first with a small amount of ethanol and then with ether. The acid was regenerated by addition of 10% sulfuric acid solution (250 cc.) to a suspension of the potassium salt in ethyl ether (600 cc.) and methanol (30 cc.). The ether extract was washed with water, dried over sodium sulfate, and the solvent removed by distillation to yield purified C-diacid (23.1 g., $E(1\%, 1\text{ cm.})(332\ m\mu)$ 875). This was then crystallized from acetone; m.p. 192° (with decarboxylation); $\epsilon(332\ m\mu)$ 31,800.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.2; H, 8.2. Found: C, 72.6; H, 8.5.

The bis-*n*-butylamine salt was prepared by the method described by Cawley[25] for the corresponding derivative of the diacid from cinnamaldehyde; m.p. 141°.

Anal. Calcd. for $C_{29}H_{50}O_4N_2$: N, 5.7. Found: N, 5.9.

D-Diacid.—The condensation of *cis*-β-ionylideneacetaldehyde (4 g.) with methyl β-methylglutaconate (3.3 g.) by the method described for the *trans* isomer gave 5.6 g. of D-diacid with $E(1\%, 1\text{ cm.})(328\ m\mu)$ 686. This was also crystallized from acetone, m.p. 190.5° (with decarboxylation); $\epsilon(326\ m\mu)$ 24,500.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.2; H, 8.2. Found: C, 73.6; H, 8.4.

Unlike the C-diacid, this acid was soluble in alcoholic potassium hydroxide under the conditions used for purifying the C-isomer.

4-Carboxy-α-vitamin A Acid.—From the reaction of α-ionylideneacetaldehyde (13.5 g.) with methyl β-methylglutaconate (11 g.), a concentrate of the 4-carboxy-α-vitamin A acid was obtained as a yellow solid (13.5 g., $E(1\%, 1\text{ cm.})$ (319 mμ) 887).

Vitamin A Acids.[38] **2-*cis*- or Neovitamin A Acid.**—A solution of crystalline C-diacid (15 g.) in 2,4-lutidine (75 cc.) containing copper acetate (0.075 g.) was heated at 120–125° with stirring for 2 hours during which a steady evolution of carbon dioxide occurred. After cooling, the solution was diluted with ethyl ether (400 cc.) and washed successively with 10% sulfuric acid to remove the 2,4-lutidine. The ether layer was then extracted with five portions of 0.5 *N* aqueous potassium hydroxide (200 cc. each) and the combined alkaline extracts acidified by the addition of 10% sulfuric acid solution (300 cc.). The acid solution was then extracted twice with ethyl ether (500 cc. each) and the combined ether extracts washed three times with 10% sodium sulfate solution, dried over anhydrous sodium sulfate, and the solvent removed under reduced pressure. The concentrate of neovitamin A acid (10.7 g., $E(1\%, 1\text{ cm.})$ (351 mμ) 1075) was crystallized from isopropyl alcohol (75 cc.) to give purified neovitamin A acid (6 g., reddish orange plates, m.p. 174–175°; $\epsilon(354\ m\mu)$ 39,800).

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 80.0; H, 9.4. Found: C, 79.8; H, 9.1.

The methyl ester was prepared by the same method used to esterify the β-ionylideneacetic acids. It crystallized from methanol at 0° as yellow needles, m.p. 66–67°; $\epsilon(359\ m\mu)$ 38,400. A polymorphic form obtained on one occasion melted at 48–49°.

2,6-Di-*cis*-vitamin A Acid.—Decarboxylation of D-diacid (4.0 g.) by the method described for C-diacid gave a concentrate of 2,6-di-*cis*-vitamin A acid (3.3 g., $E(1\%, 1\text{ cm.})$ (345 mμ) 843). This crystallized as yellow, transparent crystals from acetonitrile, m.p. 135–6°; $\epsilon(346\ m\mu)$ 34,500.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 80.0; H, 9.4. Found: C, 79.8; H, 9.2.

The methyl ester was prepared, yellow oil, $\epsilon(352\ m\mu)$ 34,000.

6-*cis*-Vitamin A Acid.—A solution of 2,6-di-*cis*-vitamin A acid (1.5 g., $E(1\%, 1\text{ cm.})(346\ m\mu)$ 1150) in isopropyl ether (30 cc.) containing iodine (15 mg.) was exposed to the light from a 60-watt frosted lamp for 1 hour which caused precipitation of a copious yellow solid. After evaporation of approximately half of the isopropyl ether under a stream of nitrogen, the precipitate was collected by filtration. On further evaporation of the filtrate a second crop of solids was obtained. The combined precipitates were recrystallized from ethanol at 25° to give 6-*cis*-vitamin A acid, 1.06 g., yellow crystals, m.p. 189–191°; $\epsilon(345\ m\mu)$ 36,900.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 80.0; H, 9.4. Found: C, 79.1; H, 9.1.

The methyl ester was prepared, m.p. 36–38°, $\epsilon(348\ m\mu)$ 35,400.

All-*trans*-vitamin A Acid (from Neovitamin A Acid).—A solution of crystalline neovitamin A acid (1.4 g., m.p. 173–174°) in a mixture of benzene (14 cc.) and ethyl ether (14 cc.) containing iodine (14 mg.) was allowed to stand at 25° in laboratory light for 6 hours, then cooled to −15° to cause crystallization. The yellow solids were filtered and recrystallized twice from ethyl alcohol to give the all *trans*-vitamin A acid as yellow needles, m.p. 179-180°; $\epsilon(350\ m\mu)$ 45,200.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 80.0; H, 9.4. Found: C, 80.1; H, 9.8.

The methyl ester was prepared in two polymorphic forms, m.p. 56–56.5°, 72–73°; $\epsilon(354\ m\mu)$ 44,400.

All-*trans*-vitamin A Acid (from 2,6-Di-*cis*-vitamin A Acid Methyl Ester.—To a solution of the methyl ester of 2,6-di-*cis*-vitamin A acid (1.0 g.) in acetonitrile (14 cc.) was added an acetonitrile–hydrochloric acid solution (1.2 cc., 0.6 *N*) which had been made by bubbling dry hydrogen chloride gas through the acetonitrile. After standing for 30 minutes at 25°, the dark colored solution was diluted with water (60 cc.) and extracted with ether. After washing the ether extract with saturated sodium bicarbonate solution, and finally with water, it was dried over anhydrous sodium sulfate, filtered and evaporated under a stream of nitrogen.

The residue (0.99 g.) which contained an appreciable amount of the retroionylidene isomer as evidenced by the triple peak in its ultraviolet absorption spectrum, $E(1\%, 1\text{ cm.})(334, 347, 365\ m\mu)$ 1045, 1315, 1130, was dissolved in alcoholic potassium hydroxide (30 cc., 2 *N*) and refluxed 1 hour. After dilution with water (100 cc.), the alkaline solution was extracted with ether to remove unsaponifiable impurities, then acidified with 10% sulfuric acid. The acids were extracted with ether.

After washing the ether extract with water, it was dried over sodium sulfate, filtered and evaporated. The solid orange residue (0.53 g.) was crystallized from methanol (3.5 cc.) yielding yellow crystals (0.14 g.) of all-*trans*-vitamin A acid, m.p. 180–181°, $\epsilon(350\ m\mu)$ 44,300. A mixed melting point with the all-*trans* acid prepared by isomerization of neovitamin A acid with iodine showed no depression.

α-Vitamin A Acid.—Decarboxylation of 4-carboxy-α-vitamin A acid (12 g.) by the method described for the decarboxylation of C-diacid gave a concentrate of the corresponding α-vitamin A acid, $E(1\%, 1\text{ cm.})(340\ m\mu)$ 1100.

Karrer, *et al.*,[39] have prepared this acid by another method. They reported it to have λ_{max} 342 mμ but gave no extinction coefficient.

Vitamin A Isomers.—The general method described above for the reduction of the methyl β-ionylideneacetates was used except that the reaction temperature was lowered to −50° to avoid isomerization of the vitamin A during the reduction step. The vitamin A alcohols obtained were purified by low temperature crystallization from ethyl formate as described previously.[40]

The acetate and *p*-phenylazobenzoate esters of the crystalline vitamin A isomers were also prepared and purified by methods earlier described.[18]

From crystalline neovitamin A acid methyl ester (3.9 g.) was obtained a concentrate of neovitamin A, 3.6 g., $E(1\%, 1\text{ cm.})(328\ m\mu)$ 1550. Properties of crystals are listed in Table I.

The mixed melting point of the *p*-phenylazobenzoate ester

(38) The alcohol used as solvent in the ultraviolet absorption measurements made on the vitamin A acids was specially purified by adding potassium hydroxide pellets (1–2 g.) and potassium permanganate (0.5–1.0 g.) to reagent grade absolute ethanol (3 liters) and distilling through a packed column. This followed from our finding that traces of acid or alkaline impurities caused the absorption maximum and extinction coefficient to change. For example, the maximum for all-*trans*-vitamin A acid in ethanol containing a trace of alkali carbonate was shifted from 350 to 337 mμ with an increase in extinction coefficient from 1507 to 1750.

(39) P. Karrer, E. Jucker and E. Schick, *Helv. Chim. Acta*, **29**, 704 (1946).

(40) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2411 (1942).

with natural neovitamin A *p*-phenylazobenzoate (m.p. 94–96°) was undepressed (94.5–96°), acetate, oil, ϵ(328 mμ) 47,000.

From all-*trans*-vitamin A acid methyl ester (3.5 g., made from the crystalline all-*trans* acid isolated from the isomerate of neovitamin A acid) was obtained a concentrate of all-*trans*-vitamin A, 3.3 g., E(1%, 1 cm.)(326 mμ) 1620; properties of crystals, Table I; acetate ester, m.p. 57.5–58°, ϵ(326 mμ) 51,200.

From crystalline 2,6-di-*cis*-vitamin A acid methyl ester (0.6 g.) was obtained a concentrate of the di-*cis*-vitamin A alcohol, 0.53 g., E(1%, 1 cm.)(324 mμ) 1210; properties of crystals, Table I; acetate, oil, ϵ(324 mμ) 36,500.

From crystalline 6-*cis*-vitamin A acid methyl ester (0.52 g.) was obtained a concentrate of 6-*cis*-vitamin A alcohol, 0.47 g., E(1%, 1 cm.)(323 mμ) 1412; properties of crystals, Table I; acetate, oil, ϵ(323 mμ) 39,400.

α-Vitamin A.—The concentrate of α-vitamin A acid (12 g.) was directly reduced using conditions described for the reduction of *cis*-β-ionylideneacetic acid. No precautions were taken, such as lowering the reaction temperature, to avoid isomerization during this reduction. A mixed isomer concentrate of α-vitamin A (10 g.) was obtained having E(1%, 1 cm.)(300, 312, 326 mμ) 907, 1165, 1100. When further purified by adsorption on a column of sodium silicoaluminate,[34] a fraction was obtained (3.5 g.) which had E(1%, 1 cm.) (298, 311, 325 mμ) 1220, 1650, 1500. Although the isomer composition of this purified fraction was not determined, indirect evidence based on properties of the crystalline aldehyde prepared from it[9] suggested that it contained a substantial amount of the all-*trans* isomer.

Reactions with Maleic Anhydride.—The rate of reaction of the *p*-phenylazobenzoates of the four vitamin A isomers with maleic anhydride was determined under the conditions described earlier for neovitamin A.[18] A plot of the values, obtained by assaying the reaction mixtures at various time intervals by the antimony trichloride method, is shown in Fig. 6.

The ester of 2,6-di-*cis*-vitamin A, like that of neovitamin A, reacted slowly while 6-*cis*-vitamin A *p*-phenylazobenzoate, like the all-*trans* isomer, reacted rapidly (recovery only 3% after 18 hours).

Substitution of diethyl ether for benzene as the reaction solvent did not greatly alter the rate of reaction with maleic anhydride.

Isolation of Maleic Anhydride-*trans*-Vitamin A Acetate Adduct.—To a solution of crystalline all-*trans*-vitamin A acetate (2.4 g.) in anhydrous ethyl ether (200 cc.) was added a solution of maleic anhydride (100 g.) in ethyl ether (800 cc.). The solution was allowed to stand at 25° in the dark for 40 hours and then washed with eight portions (250 cc. each) of 0.5 *N* aqueous potassium hydroxide solution, two portions (250 cc. each) of dilute sulfuric acid, and finally with distilled water, until the washings were neutral. After drying the ether solution over anhydrous sodium sulfate and filtering, the solvent was removed by distillation. The residual oil, which had E(1%, 1 cm.)(237, 261 mμ) 344, 350 was dissolved in petroleum ether (50 cc.) and the solution cooled to 0°. This caused oiling out of the product.

After decanting the solvent, the oil was dissolved in ethyl ether (5 cc.) and diluted with more petroleum ether (75 cc.). Slow evaporation to a volume of approximately 20 cc. caused precipitation of the adduct as fine white crystals (1.1 g.) which were then recrystallized from petroleum ether, m.p. 96–96.6°; ϵ(238, 261 mμ) 12,900, 13,400.

Anal. Calcd. for $C_{26}H_{34}O_5$: C, 73.2; H, 8.0. Found: C, 73.4; H, 8.3.

When the experiment was repeated on a sample of 6-*cis*-vitamin A acetate (0.053 g.), the product had ϵ(234, 265 mμ) 12,500, 12,600. This product was not isolated as crystals.

Isomerization Experiments (Vitamin A Esters).—A solution of synthetic neovitamin A acetate (1 g.) in petroleum ether (2 cc.) containing iodine (0.5 mg.) was allowed to stand for 2 hours at 25°, protected from light. The solution after dilution with more petroleum ether was then washed with dilute sodium thiosulfate solution and water, dried over anhydrous sodium sulfate, and evaporated in a stream of nitrogen. The residue (0.99 g.) had E(1%, 1 cm.) (326 mμ) 1440 and assayed for 32% neovitamin A acetate by the maleic anhydride method. It was then dissolved in a 2:1 methanol:ethyl formate mixture (6.5 cc.) and cooled to −20° for 2 days. The crystals (0.18 g.) of all-*trans*-vitamin A acetate were filtered off and recrystallized at −20°, m.p. 58.5–59°, ϵ(326 mμ) 51700.

A solution of 2,6-di-*cis*-vitamin A *p*-phenylazobenzoate (0.2 g.) in benzene (50 cc.) containing iodine (0.2 mg.) was stored at 25° in the dark for 2 hours as above. After removing the iodine by passing the solution through a layer of powdered sodium thiosulfate, the solvent was removed by evaporation under a stream of nitrogen. A sample of the residual red viscous oil then assayed for no more than 31.5% of the original di-*cis* isomer by the maleic anhydride test.

After dissolving the residue in isopropyl alcohol (4 cc.) and cooling to 0° a crop of crystals was obtained, m.p. 74–76°. No depression of melting point was observed when mixed with 6-*cis*-vitamin A phenylazobenzoate. However, the melting point of a mixture of the product and all-*trans*-vitamin A *p*-phenylazobenzoate was depressed to 66°.

Acknowledgment.—The authors wish to express their indebtedness to Mr. Jacque Lindsay for valuable assistance in the purification of the 4-carboxyvitamin A acids and to Mr. John Dieterle for valuable experimental work in studies on isomerization and maleic anhydride reactions of the vitamin A isomers. Special acknowledgment is made to Dr. James G. Baxter and Dr. Norris D. Embree for their direction of this research.

ROCHESTER, N. Y.