Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36. Found: C, 76.51; H, 9.61.

The *acetate* of VIIIa prepared in the usual manner (pyridine–acetic anhydride) afforded needles from acetone-hexane of m.p. 212–215°. It agreed in melting point, mixture melting point, and infrared spectrum, with an authentic specimen of the compound prepared in another manner.[17]

3β,16β-Dihydroxybisnorallocholanic acid 22→16-lactone (VIIIb). The cyanoamide, IVb (100 mg.), was refluxed with 10% potassium hydroxide in methanol (30 ml.) for 6 hr. The product was worked up in the same manner as described above for VIIIa. It crystallized as needles (62 mg.) from aqueous ethanol and melted at 232–236°. Recrystallization raised the m.p. to 235–236°, $[\alpha]_D^{20}$ −43° (chloroform). The melting point, infrared spectra, and rotation agreed with an authentic sample of tigogenin lactone.[14]

23-Oximinosolasodine (VIa). IIIa (360 mg.) was refluxed with 10% potassium hydroxide in methanol (100 ml.) for 7 hr. After removal of the methanol *in vacuo*, water was added and the precipitate collected by centrifugation. The compound crystallized from methanol yielding 256 mg. (85%) of plates m.p. 180–183°, $[\alpha]_D^{20}$ −93° (chloroform), $\lambda_{max}^{C_2H_5OH}$ 231 mμ (log ε, 3.63).

Anal. Calcd. for $C_{27}H_{42}O_3N_2$: C, 73.26; H, 9.56; N, 6.33. Found: C, 73.33; H, 9.67; N, 6.26.

When the compound was recrystallized from acetone-hexane, needles which melted at 194–198° were obtained. If this substance was recrystallized from ethyl alcohol, needles of m.p. 161–164° were formed

Anal. Calcd. for $C_{27}H_{42}O_3N_2 \cdot {}^1/_2C_2H_5OH$: C, 72.22; H, 9.72; N, 6.01. Found: C, 71.90; H, 10.04; N, 5.87.

The compound of m.p. 180–183° was converted into the substance of m.p. 161–164° when recrystallized from ethanol.

23-Oximinotomatidine (VIb). Three hundred milligrams of the oximinopseudo compound IIIb, was refluxed for 7 hr. with 80 ml. of a solution of 10% potassium hydroxide in methanol. The product which crystallized from slightly moist methanol as needles melted at 207–209°, $[\alpha]_D^{20}$ −54° (chloroform), $\lambda_{max}^{C_2H_5OH}$ 231 mμ (log ε, 3.70). The substance is a hydrate as shown by the analyses.

Anal. Calcd. for $C_{27}H_{44}O_3N_2 \cdot H_2O$: C, 70.09; H, 10.02; N, 6.06. Found: C, 70.00; H, 10.16; N, 5.89.

After the compound was dried at 150° for 20 hr. *in vacuo*, the m.p. rose to 234–237°.

Anal. Calcd. for $C_{27}H_{44}O_3N_2$: C, 72.93; H, 9.97; N, 6.30. Found: C, 72.90; H, 10.27; N, 6.60.

Imino nitrile, VIIa. A solution of 180 mg. of VIa in 7 ml. of pyridine and 2 ml. of acetic anhydride was allowed to stand overnight at 5° and poured on ice. The product which crystallized from ether-hexane yielded 152 mg. (80%) of needles, m.p. 145–148°. An analytical sample recrystallized from the same solvent system melted at 147–150°, $[\alpha]_D^{20}$ −6.5° (chloroform), $\lambda_{max}^{CHCl_3}$ 4.55 μ (—C≡N); 5.78 μ (—OAc) 5.88 μ[18] (—C=N—).

Anal. Calcd. for $C_{29}H_{42}O_3N_2$: C, 74.64; N, 9.07; N, 6.00. Found: C, 74.76; H, 9.37; N, 6.20.

Imino nitrile, VIIb. One hundred milligrams of VIb in 1 ml. of acetic anhydride and 3 ml. of pyridine was treated in the same manner as described above for VIIa. The product which was crystallized from hexane yielded 86 mg. of prisms of m.p. 126–128°, $[\alpha]_D^{20}$ +1.7° (chloroform); $\lambda_{max}^{CHCl_3}$ 4.55 μ (C≡N); 5.80 (OAc); 5.88 μ (—C=N—).

Anal. Calcd. for $C_{29}H_{44}O_3N_2$: C, 74.32; H, 9.46; N, 5.98. Found: C, 74.47; H, 9.56; N, 6.17.

3β-Acetoxy-16β-hydroxy-5-bisnorcholenic 22→16-lactone (IXa) *from the imino nitrile*, VIIa. A solution of 65 mg. of VIIa in 10 ml. of 90% acetic acid was refluxed for 30 min. and the solvent removed *in vacuo*. The residue was dissolved in methylene chloride and washed with 1*N* sodium bicarbonate solution. After removal of the solvent, the residue, which crystallized from methanol, yielded 49 mg. IXa, m.p. 208–212°. By recrystallization from acetone-hexane, plates of m.p. 212–215° were obtained. The melting point, mixture melting point, and infrared spectra were identical with that of the specimen prepared from the acetylation of the hydrolysis of the cyanoamide, IVa.

3β-Acetoxy-16β-hydroxybisnorallocholanic 22→16-lactone (IXb) *from the imino nitrile*, VIIb. VIIb (48 mg.) was treated in the same manner as reported above for the preparation of IXa. Needles (35 mg.) of m.p. 210–214° were obtained from aqueous ethanol. Recrystallization from the same solvent raised the m.p. to 216–218°, $[\alpha]_D^{20}$ −48° (chloroform). Its properties (melting point, mixture melting point, infrared spectra, and rotation) were in agreement with an authentic specimen of the acetate of tigogenin lactone.

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[CONTRIBUTION FROM THE ARGONNE NATIONAL LABORATORY]

Spectra of Eschscholtzxanthin and Other Carotenoid Pigments

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The spectra in the visible region and the chromatographic behavior show that the twelve double bonds of eschscholtzxanthin occur in one conjugated system. The infrared spectra indicate that this xanthophyll is a derivative of *sym*-dehydro-β-carotene (dehydroretrocarotene) with a central single bond.

In 1938, eschscholtzxanthin, a remarkably labile dihydroxy carotenoid with twelve all-*trans* double bonds, was isolated from the golden yellow petals of the California poppy.[1] In 1948 Karrer and Jucker[2] proposed that eschscholtzxanthin is a dihydroxy-γ-carotene even though the absorption maxima of γ-carotene occur at wave lengths some 10 mμ shorter than those of the poppy xanthophyll.[1-3] In 1951, Karrer and Leumann[4] postulated

(1) H. H. Strain, *J. Biol. Chem.*, **123**, 425 (1938).

(2) P. Karrer and E. Jucker, *Carotinoide*, Birkhäuser, Basel, 1948.

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that eschscholtzxanthin is a dihydroxy derivative of a *sym*-dehydro-β-carotene with twelve symmetrically arranged, conjugated double bonds and, unlike most carotenoids, with a single bond at the center of the molecule.[2,3]

Isler and collaborators have reported the visible and infrared absorption spectra of various modified carotenoids with total unsaturation equal to twelve double bonds. Pigments with a single bond at the center of the molecule[5] (as 7,7′-dihydro-β-carotene and dehydroretrocarotene, presumably *sym*-dehydro-β-carotene) exhibit three carotene-like maxima in the visible spectrum, but they show two maxima in the region 950–1000 cm.$^{-1}$ where most carotenoids exhibit but one maximum.[6] Pigments with twelve conjugated double bonds but with a single bond at the center of the molecule (as 3,4-monodehydro-β-carotene) exhibit only one maximum in the visible region but yield an infrared curve almost identical with that of the common carotenes.[7] Pigments with a triple bond at the center of the molecule (as dehydroisozeaxanthin[8] and dehydrocryptoxanthin[9]) exhibit maxima in the visible region some 20 mμ shorter than those of β-carotene and yield an infrared curve very similar to that of the common carotenes.

To correlate the spectral properties of eschscholtzxanthin with the structural features of other carotenoids, we have now compared the absorption spectra of this xanthophyll and its acetate with the spectra of the more common carotenoids. We have also compared the chromatographic behavior of all these substances.

Materials. Most of the eschscholtzxanthin, its acetate, the lutein, the zeaxanthin, and the cryptoxanthin employed in these studies had been isolated in 1934.[1,3] Fresh preparations and recrystallized preparations were spectroscopically and chromatographically identical with the preparations stored in evacuated and sealed glass tubes.

Absorption in the visible spectrum. The molecular spectral absorption curve of eschscholtzxanthin diacetate, which is identical with that of eschscholtzxanthin,[1] is compared with the curve of lycopene and with the wave lengths of the principal absorption maxima of α-carotene, β-carotene, γ-carotene, and dehydro-β-carotene in Fig. 1. A more extensive comparison of the spectral properties of several carotenes and their hydroxy derivatives, provided by Table I, shows that the hydroxy compounds and their esters exhibit maximum absorption at the same wave lengths as those of the parent carotenes.

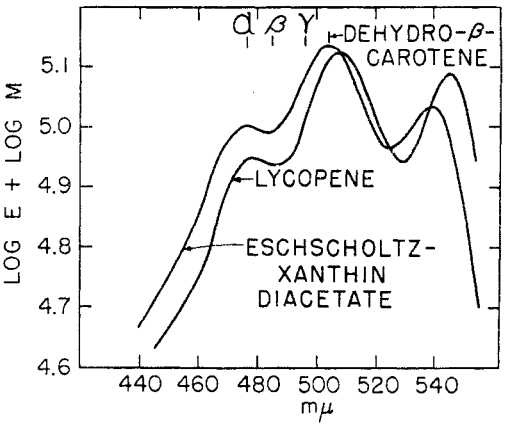

Fig. 1. Spectral absorption curves of eschscholtzxanthin diacetate and of lycopene in carbon disulfide. Principal absorption maxima of α-, β-, γ-, and *sym*-dehydro-β-carotene are indicated

TABLE I

ABSORPTION MAXIMA OF VARIOUS CAROTENOIDS[1-4]

(Brackets indicate pigments with the same chromophoric polyene structure)

Carotenoid	Double Bonds	—OH Groups	Wave Lengths of Abs. Max. (mμ)		(CS₂)
Dehydrolycopene	15	0	520	557	601
Lycopene	11 + 2	0	477	507.5	548
Lycophyll	11 + 2	2	472	506	546
Lycoxanthin	11 + 2	1	472	506	546
sym-Dehydro-β-carotene	12	0	472	504	543
Eschscholtzxanthin	12	2	475	502	536
Eschscholtzxanthin acetate	12	2[a]	475	502	536
γ-Carotene	11 + 1	0	463	496	533.5
Rubixanthin	11 + 1	1	461	494	533
β-Carotene	11	0	450	485	520
Zeaxanthin	11	2	450	482	517
Zeaxanthin acetate	11	2[a]	450	482	519
Cryptoxanthin	11	1	452	483	519
α-Carotene	10 + 1	0	—	477	509
Lutein	10 + 1	2	445	475	508

[a] Acetate groups.

The wave lengths of the absorption maxima in carbon disulfide (Table I) and in petroleum ether and chloroform (Table II) indicate very similar spectra for dehydro-β-carotene and eschscholtzxanthin.

Absorption in the infrared. All the infrared absorption measurements were made with a calibrated Beckman IR-7 recording spectrophotometer. The carotenoids were examined under the same conditions employed with the fully deuterated

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(6) K. Lunde and L. Zechmeister, *J. Am. Chem. Soc.*, **77**, 1647 (1955).

(7) O. Isler, H. Lindlar, M. Montavon, R. Rüegg, and P. Zeller, *Helv. Chim. Acta*, **39**, 274 (1956).

(8) O. Isler, H. Lindlar, M. Montavon, R. Rüegg, and P. Zeller, *Helv. Chim. Acta*, **39**, 449 (1956).

(9) O. Isler, H. Lindlar, M. Montavon, R. Rüegg, G. Saucy, and P. Zeller, *Helv. Chim. Acta*, **40**, 456 (1957).

TABLE II

ABSORPTION MAXIMA OF LYCOPENE, ESCHSCHOLTZXANTHIN, DEHYDRO-β-CAROTENE, AND γ-CAROTENE IN PETROLEUM ETHER AND CHLOROFORM[1-5]

Carotenoid	Wave Lengths of Abs. Maxima (mμ)			Solvent
Lycopene	447	475.5	506	Petroleum Ether
Eschscholtzxanthin	446	472	502	—
sym-Dehydro-β-carotene	447	475	504	—
Dehydroretrocarotene	444	471	502	—
γ-Carotene	431	462	495	—
Lycopene	453	480	517	Chloroform
Eschscholtzxanthin	456	484	513	—
sym-Dehydro-β-carotene	455	485	518	—
γ-Carotene	446	475	508.5	—

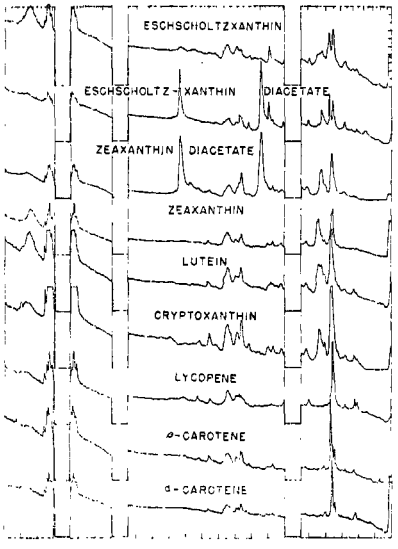

Fig. 2. Infrared absorption spectra of carotenoid pigments in potassium bromide pellets

carotenoids.[10] They were compressed with potassium bromide powder (0.5% carotenoid), melted between potassium bromide plates, dissolved in various solvents such as carbon tetrachloride, bromoform, deuterochloroform, and tetrachloroethylene, and mulled with fluorocarbons.

The infrared curves obtained with bromide powder (Fig. 2) indicate much light scattering at the short wave lengths. This effect frequently interfered with the detection of the absorption band of the associated hydroxyl groups at 3400 cm.$^{-1}$ Curves for α- and β-carotene, but not for lycopene or xanthophylls, exhibited an absorption maximum at 947 cm.$^{-1}$, not present in the curves for the melts or for the solutions. Previously observed by van Dan[11] with suspensions, this band of the crystalline α- and β-carotene must be attributed to the molecular aggregation in the crystals.[12]

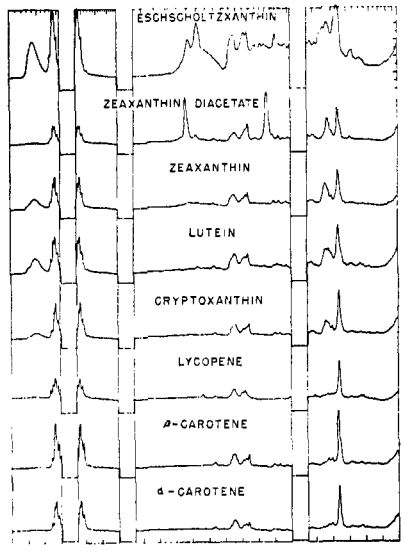

Fig. 3. Infrared absorption spectra of carotenoid pigments after melting between potassium bromide plates

Curves determined with melts (Fig. 3) were not affected by light scattering or by the crystal structure. The eschscholtzxanthin curve was altered to a much greater extent than the curves of the other carotenoids when the pigments were melted, usually for only thirty-five seconds. The changes resulted in the coalescence of the peaks at 955 and 977 cm.$^{-1}$ and in the enhancement of the maxima at 1665 and 1718 cm.$^{-1}$ The eschscholtzxanthin acetate could not be melted without extensive decolorization and decomposition.[1]

Curves determined in solvents were very similar to those observed with melts. They agreed with those reported by Lunde and Zechmeister,[6] who included a curve for γ-carotene. In carbon tetrachloride, the absorption by the free hydroxyl group of xanthophylls was moderately strong and very sharp at 3644 cm.$^{-1}$ The broad maximum at 3400 cm.$^{-1}$, due to the associated hydroxyl groups, was barely detectable.[13,14] In melts and in crystals, there was a pronounced maximum at 3400 cm.$^{-1}$ but none at 3644 cm.$^{-1}$

Except for the absorption by the associated hydroxyl groups in eschscholtzxanthin (3400 cm.$^{-1}$) and that by the ester groups in the acetate (1735 and 1245 cm.$^{-1}$), the spectra of the poppy xanthophyll and its acetate are basically alike. Both pigments, in powders and in bromoform, exhibit two prominent maxima at 955 cm.$^{-1}$ and 977 cm.$^{-1}$ whereas the other carotenoids exhibit only one maximum at 965 cm.$^{-1}$, the so-called all-*trans* peak.[6] Both exhibit indistinct maxima at 1665 cm.$^{-1}$ and 1718 cm.$^{-1}$ not apparent in the curves of the other carotenoids. Both exhibit relatively greater absorption near 2970 cm.$^{-1}$ than that

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(11) N. van Dan, *Naturwissenschaften*, **46**, 18 (1959).
(12) A. W. Baker, *J. Phys. Chem.*, **61**, 450 (1957).
(13) S. Liaaen Jensen, *Acta Chem. Scand.*, **13**, 842 (1959).
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exhibited by the common carotenoids, as shown by Figs. 2 and 3.

Many of the infrared absorption maxima of eschscholtzxanthin can be correlated with the maxima of the common carotenoids and in this way with particular structural groups.[15] Maxima at 2960 and 2870 cm.$^{-1}$ are due to methyl groups; that at 3030 to —CH=CH—; that at 2926 to —CH_2—; those at 1450 and 1375 to CH_3C; those at 1380 and 1365 to $(CH_3)_2C$; that at 1030 to C—O.

Chromatoyraphy of eschscholtzxanthin and eschscholtzxanthin diacetate. In columns of activated magnesia, which attracts double bonds as well as hydroxyl groups,[3,16,17] and with petroleum ether (b.p. 20–40°)-25% acetone as solvent, eschscholtzxanthin formed a purple-orange zone above zeaxanthin, neoxanthin, and lycopene. In sugar columns, which do not attract double bonds,[16,17] and with petroleum ether plus benzene, the eschscholtzxanthin was sorbed with violaxanthin below neoxanthin and above zeaxanthin plus lutein. In sugar columns with petroleum ether plus 0.5% *n*-propyl alcohol, the eschscholtzxanthin was sorbed below violaxanthin, above zeaxanthin plus lutein, and just above chlorophyll *b*.[16]

In magnesia columns and with petroleum ether plus 25% acetone, eschscholtzxanthin acetate was sorbed with zeaxanthin and lycopene, far above lutein acetate. In sugar columns with petroleum ether plus 0.5% *n*-propyl alcohol, the acetate was weakly sorbed forming a pink-yellow zone, not separated from lutein acetate and separated incompletely below cryptoxanthin.

(15) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd Ed., Methuen, London; Wiley, New York, 1958.

(16) H. H. Strain, *Chloroplast Pigments and Chromatographic Analysis*, 32nd Annual Priestly Lectures, Pennsylvania State University, University Park, 1958.

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DISCUSSION

The visible spectra and the chromatographic sequences show that eschscholtzxanthin contains twelve conjugated double bonds. Most of the infrared absorption maxima of eschscholtzxanthin are similar to those of the common carotenoids and have been related to absorption by particular structural units characteristic of these substances. The most striking differences between the infrared spectra of eschscholtzxanthin and those of the common carotenoids are the two bands of the former, at 955 cm.$^{-1}$ and 977 cm.$^{-1}$, and the single band of the latter, at 965 cm.$^{-1}$ This indicates that eschscholtzxanthin is a derivative of *sym*-dehydro-β-carotene (dehydroretrocarotene)[4,5] with a single band at the center of the molecule. It shows that this xanthophyll cannot be a derivative of γ-carotene.[6]

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[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE EDWARD DANIELS FAULKNER ARTHRITIS CLINIC, PRESBYTERIAN HOSPITAL]

The Structure of Keratosulfate of Bovine Cornea[1]

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The structure of keratosulfate from bovine cornea has been studied by methylation techniques. Hydrolysis products from methylated, sulfated, and desulfated polymers establish the disaccharide, *O*-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-D-glucose 6-sulfate) linked (1 → 3) to the D-galactose of the next recurrent disaccharide unit, as the repeating sequence in keratosulfate.

Keratosulfate, initially isolated from cornea[2] and recently reported from diverse sources,[3] is distinguished from the other connective tissue mucopolysaccharides by the absence of uronic acid and the presence of galactose. Its atypical nature is further characterized by its resemblance to blood group substances, exemplified by: 1) the presence of small amounts of easily hydrolyzable methylpentose, 2) its cleavage by blood group substance-cleaving enzymes in contrast to its inertness toward hyaluronidases and chondrosulfatases, and 3) the cross reaction of the desulfated polysaccharide with antiblood group sera.[4]

(1) This work was supported in part by grants from the National Science Foundation and the U. S. Public Health Service.

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