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162. Nobuo Suzuki and Kiyoshi Tsukida : On Some *cis*-Forms of Luteochrome.

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A number of carotenoid epoxides and furanoid oxides are known as naturally occurring substances,[*2] some of which are regarded as very important intermediates for carotenoids biosynthesis1) and have acquired increasing biological significance because of their provitamin-A effect.2) These epoxides have the characteristic feature of forming the corresponding furanoid oxides by contacting with acid3) or alkali4) under mild conditions.

On the reaction of β-carotene (I) with perphthalic acid, β-carotene mono-epoxide (II) and di-epoxide (III) are formed together with furanoid oxides such as mutatochrome (IV), aurochrome (V), and luteochrome (VI)3),4) (Fig. 1). Mutatochrome and aurochrome were detected or isolated in several higher plants,[*3],1,5~7) while only a trace of luteochrome was detected in the berries of *Pyracantha flava*.8)

One of the authors had reported earlier the stereoisomerization of β-carotene epoxides and the simultaneous formation of furanoid oxides.4) In the present paper a quantitative and configurational studies are presented concerning some *cis*-forms of luteochrome (β-carotene monoëpoxide monofuranoid oxide), one of the furanoid oxides described above.

Several stereoisomerization treatments applied generally to cause *trans*—*cis* isomerization of polyenes, such as refluxing, illumination, insolation, and iodine catalysis of a solution, or melting crystals, converted all-*trans* luteochrome into the expected stereoisomeric equilibrium mixtures and aurochrome. This complicated reaction product was resolved chromatographically and four *cis* zones, listed in Tables I and II, appeared on the column.

All-*trans* compound was regenerated from each isomer on the column and identified

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[*2] Fourteen carotenoid epoxides and 15 furanoid oxides are known. cf. P. Karrer, E. Jucker: "Carotenoids," Elsevier Publishing Co., New York, 1950; G. Mackinney : Ann. Rev. Biochem., **21**, 473(1952); T.W. Goodwin : *Ibid.*, **24**, 497(1955); "Carotenoids—Their Comparative Biochemistry," Chem. Publ. Co., New York, 1954; "Moderne Methoden der Pflanzenanalyse," Vol. 3, 272(1955), Springer-Verlag, Berlin.

[*3] Aurochrome was found in the tubers of Irish potato,7) petals of *Calendula officinalis*,6) red fruits of red pepper,1) and berries of *Cotoneaster bullata* and *Hippophae rhamnoides*.8) Mutatochrome (=citroxanthin) was detected or isolated from the orange peel,5) petals of *Calendula officinalis*,6) fruits and leaves of red pepper,1) and berries of *Cotoneaster bullata*, *C. frigida*, *C. hebephylla*, *Crataegus oxyacantha*, *C. pratensis*, *Pyracantha flava*, *Rosa moyesii*, *Sorbus aucuparia*, *Taxus baccata* and *Viburnum opulus*.8)

1) L. Cholnoky, C. Gyoergyfy, E. Nagy, M. Panczel : Acta Chim. Acad. Sci. Hung., **6**, 143(1955); Nature, **178**, 410(1956).

2) P. Karrer, E. Jucker, J. Rutschmann, K. Steinlin : Helv. Chim. Acta, **28**, 1146(1945); H. v. Euler, P. Karrer : *Ibid.*, **33**, 1481(1950); N.T. Gridgeman, R.F. Hunter, N.E. Williams : J. Chem. Soc., **1947**, 131.

3) P. Karrer, E. Jucker : Helv. Chim. Acta, **28**, 427(1945); cf., P. Karrer, E. Jucker, K. Steinlin: *Ibid.*, **29**, 233(1946); B.G. Savinov, F.L. Grindberg : Ukrain. Khim. Zhur., **16**, 358(1950) (C.A., 48, 9975).

4) K. Tsukida, L. Zechmeister : Arch. Biochem. Biophys., **74**, 408(1958).

5) P. Karrer, E. Jucker : Helv. Chim. Acta, **27**, 1695(1944); *ibid.*, **30**, 536(1947).

6) T.W. Goodwin : Biochem. J., **58**, 90(1954).

7) B.C. Brunstetter, H.G. Wiseman : Plant Physiol., **22**, 421(1947).

8) T.W. Goodwin : Biochem. J., **62**, 346(1956). cf., R.F. Hunter, R.M. Krakenberger : J. Chem. Soc., **1947**, 1; F.H. Schwarzenbach : Helv. Chim. Acta, **34**, 1064(1951); Vierteljahrsschr. naturforsch. Ges., Zürich 98, Beih, No. 1 (1953)(C.A., **48**, 4061).

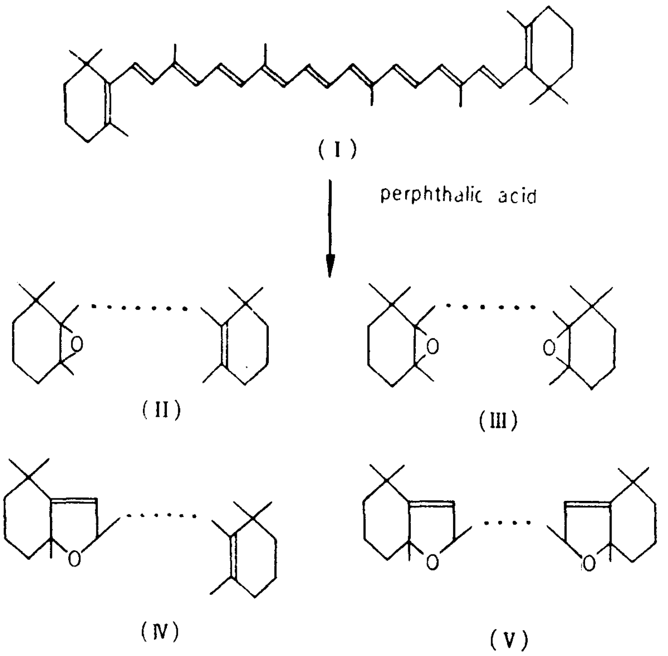
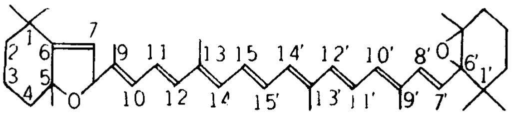


Fig. 1.



(VI)

Table I. Composition of the Mixtures of *cis-trans* Isomers and Simultaneously-formed Aurochrome obtained from All-*trans* Luteochrome[a]

Treatment	Unchanged all-*trans* form	*cis*-Form luteochrome				Aurochrome	Irreversible loss
		neo-V	neo-U	neo-A	neo-B		
I_2 in light[b]	94	0.8	trace	1	4	0.2	trace
I_2 in light[c]	60	0	40	0	trace	trace	0
Refluxing[d]	97	0	0	0	2.7	0.3	0
Illumination[e]	95.6	0.6	0	1.3	2.3	0.2	trace
Insolation[f]	94	1	0	1.9	3.1	trace	trace
Melting crystals[g]	57	3	0	40[h]		trace	trace

a) After chromatography, the values were obtained photometrically, in hexane solution, and refer to per cent of the starting material.

b) In a volumetric flask, the solution containing 0.34% iodine (pigment=100%) was exposed to the light from a mercury lamp (Shimazu Co., 300-w) of 12 cm. in length without any light filter, from a distance of 24 cm. for 30 min.

c) Exposed for 5 min. with 0.14% iodine under the same condition as in *b*).

d) The solution was refluxed in darkness for 1 hr.

e) The solution placed in a volumetric flask was illuminated with three 100-w Mazda bulbs symmetrically placed, from a distance of 10 cm. for 1 hr., while cooling with a fan.

f) Exposed to the direct sunshine of November for 2 hr.

g) The substance, in a thin-walled, evacuated tube, was immersed in a bath of 200° for 90 sec., then cooled in ice water.

h) Sum of neoluteochrome-B (major) and neoluteochrome-A.

with the authentic sample. The ultraviolet spectral curves of iodine-catalyzed equilibrium mixtures derived from all-*trans* form and *cis*-forms were found to be exactly identical. All-*trans* compound was found to be considerably stable to light and heat than β-carotene mono- and di-epoxides, but remarkable amount of stereoisomeric mixtures was formed when subjected to some treatments such as iodine catalysis in light

Table II. Some Spectral Characteristics of *cis-trans* Isomeric Luteochrome

Member of the set	Location of maxima in hexane (mμ)*				$E^{mol.}_{1\,cm.} \times 10^{-4}$	
					at λ_{max}	at *cis*-peak
Neoluteochrome-V	444,	*417,*	396,	312	13.5	1.0
Neoluteochrome-U	449,	*422,*	398,	312	15.3	0.8
all-*trans*	*449,*	422,	398,	313	15.7	0.8
Neoluteochrome-A	443,	*416,*	395,	313	11.9	1.5
Neoluteochrome-B	444,	*417,*	395,	313	11.1	1.7
Iodine equilibrium mixture	447,	*421,*	397,	311.5	10.3	1.2

(Listed in the order of decreasing adsorption affinities except iodine equilibrium mixture)

* The highest extinction was observed at the maximum wave-length given in italic letters.

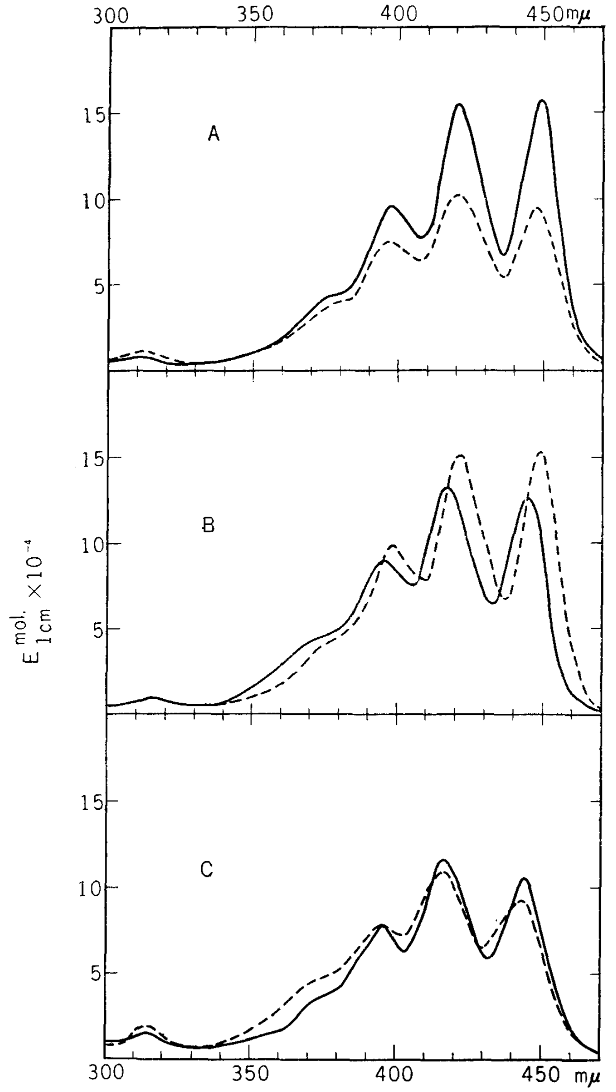


Fig. 2.

Molecular Extinction Curves of Luteochrome set in Hexane

A: all-*trans*-luteochrome, —— fresh solution; and ------ mixture of stereoisomers after iodine catalysis
B: —— neoluteochrome-V
------ neoluteochrome-U
C: —— neoluteochrome-A
------ neoluteochrome-B

or melting crystals.

The formation of neoluteochrome-V, -A, and -B caused a shift of λ_{max} toward shorter wave-lengths by only 5, 6, and 5 mμ, respectively (Fig. 2, Table II). Hence, on the basis of the work conducted by Zechmeister, *et al.*,9) these three isomers were assumed to represent mono-*cis* compounds. Neoluteochrome-U which was obtained only on

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9) L. Zechmeister: Experientia, **10**, 1(1954); Chem. Revs., **34**, 267(1944); A. Polgar, L. Zechmeister: J. Am. Chem. Soc., **64**, 1856(1942); L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgar, L. Pauling: *Ibid.*, **65**, 1940(1943).

short illumination of all-*trans* compound in the presence of iodine, showed noteworthy feature that no such shift was observed in its ultraviolet spectrum.

The spectrum of this unusual isomer was very similar to that of the parent compound but it was easily differentiated from the latter by a mixed chromatogram test. Thus, this isomer was also considered as a mono-*cis* isomer from the evidences of its infrared spectrum and of the similarity to the case of *cis*-aurochromes which exhibited no shift from the parent compound in the ultraviolet spectrum when a *cis* structure around the nearest double bond to furan ring was involved in the molecules.[4] Molecular extinction coefficients at their maximum wave-lengths and at *cis* peaks indicated that the overall molecular shape of neo-A or -B had to be a bent one while neo-U or -V might have essentially straight molecular shape.[9] Therefore, the *cis* double bond in neo-A or -B would be located near the center of the chromophore, in contrast to the most abundant *cis* forms, viz., noe-U or -V, which would possess a peripheral *cis* double bond. The latter assignment was in accordance with the location of the neo-U and -V zones above the all-*trans* zone in the chromatograms.[9]

The infrared spectra of all-*trans* and four *cis* forms are given in Fig. 3. At 7.25 μ, each isomer gave a similar but considerably stronger band than that of all-*trans* form. As this band was assigned to the typical methylated *cis* double bonds in several other stereoisomeric set of polyenes,[10] similar extent of *trans-cis* isomerization was expected for these isomers. The band at 10.45 μ in each isomer interpreted as the *trans* peak also appeared to a similar extent, but much weaker than that of all-*trans* form. Fur-

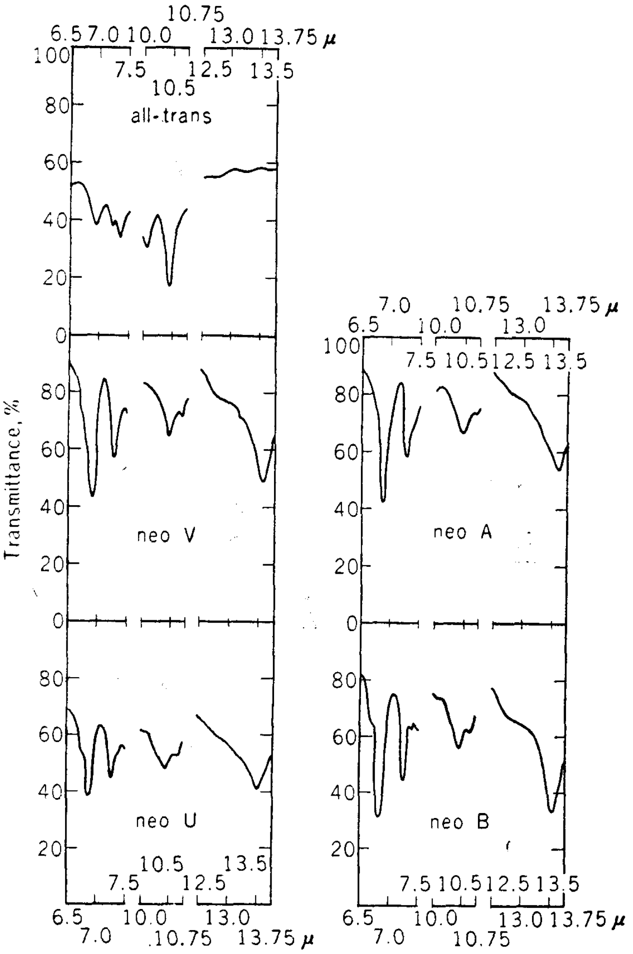

Fig. 3.

Infrared Spectra of Luteochrome Set
All-*trans* : KBr disk
Neo isomers : ca. 0.01 mm. liquid

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[10] K. Lunde, L. Zechmeister : J. Am. Chem. Soc., **77**, 1647(1955); Acta Chem. Scand., **8**, 1421(1954).

thermore, no splitting of this band into doublet or triplet and absence of absorption at 12.84~12.94 μ might exclude the 15,15′-*cis* configuration from each isomer.[10] Strong bands which appeared in the region of 13.5~13.6 μ in the spectrum of each isomer could not be explained at present time. Thus, it was concluded from infrared spectral data that all these four isomers might not have central *cis* structure, but have a similar number(s) of methylated *cis* configuration(s) in their molecules.

Luteochrome has eight double bonds which can contribute for *trans-cis* isomerization and 256 spatial configurations in all are possible if isomerization could occur at every double bond. Four *cis*-isomers which were obtained in the present experiment, exhibited characteristic fine structures in definition of their ultraviolet spectra and every ultraviolet or infrared spectral characteristics did not show any *trans-cis* isomerization around sterically hindered carbon. Hence, five double bonds, viz., 9–10, 13–14, 15–15′, 14′–13′, and 10′–9′, were found to be the effective double bonds for isomerization (cf. Fig. 1 for numbering) and 32 isomers (1 of all-*trans*, 5 of mono-*cis*, 10 of di-*cis*, 10 of tri-*cis*, 5 of tetra-*cis*, and 1 of all-*cis*) are possible.

As central-*cis* structure can be excluded as described, four *cis*-isomers which were obtained in the present work were assumed to be either one of left four mono-*cis* forms. Considering all the available experimental data and the previous work, it is possible to make a tentative proposal that neoluteochrome-A has the 13,14-mono-*cis*, neo-B the 13′-14′-mono-*cis*, neo-U the 9,10-mono-*cis*, and neo-V the 9′,10′-mono-*cis* configurations.

Experimental

Materials and Methods—All operations were carried out in dim light and the evaporation was made *in vacuo* at around room temperature. Melting points were measured in an evacuated capillary, in an electrically heated Berl block, and corrected. $Ca(OH)_2$ (Wako Pure Chem. Ind., Ltd.) was found to be the best adsorbent. The stereoisomeric mixtures could not be resolved effectively with a number of other adsorbents. Hexane-acetone (99:1) and pure acetone were used as the developer and eluant, respectively, in chromatography. Unless otherwise stated, 45×4.5 cm. column was used.

When direct weighing was impossible, because only a small amount of a *cis*-form was available in solution, the latter was iodine-isomerized and its extinction value determined at the maximum wave-length. The concentration was then calculated on the basis of the molecular extinction coefficient of the iodine equilibrium mixture as known from a parallel experiment in which a weighed all-*trans* sample had been treated with iodine. Each isomer was identified by the spectra of its own and of iodine equilibrium mixture, and by mixed chromatogram tests with other members of the set. Furthermore, all-*trans* compound was always regenerated from each isomer upon iodine treatment, followed by mixed chromatogram test with authentic all-*trans* sample. For the molecular extinction curves, Hitachi photoelectric spectrophotometer EPU-2A was used, and infrared spectra were kindly measured by Mr. Nakamachi of the Takeda Pharmaceutical Industries, Ltd.

Main source for the sample of all-*trans* luteochrome came from the corresponding zone on the chromatographic column during the course of the preparation of all-*trans*-β-carotene diepoxide and monoepoxide from all-*trans*-β-carotene.[4] Luteochrome was also obtained from β-carotene diepoxide by using the usual isomerization techniques, especially when the latter was submitted to alumina contact experiment.[4] Yellowish orange, whetstone-like crystals, m.p. 177~178°(from benzene-methanol). *Anal.* Calcd. for $C_{40}H_{56}O_2$: C, 84.43; H, 9.95. Found: C, 84.25; H, 9.99. UV λ_{max}^{hexane} mμ ($E_{1\,cm.}^{mol} \times 10^{-4}$): 448 (15.7), 422 (15.6), 397.5 (9.65), 312 (0.8).

Isomerization of all-*trans*-Luteochrome (cf. Table I)—a) Iodine Catalysis, in Light: A solution containing 10 mg. of a substance and 0.034 mg. of I_2 in 100 cc. of hexane was illuminated for 30 min. (cf. Table I) and then developed on a chromatographic column for 1 hr. The following chromatogram was obtained:

Width of zone, (mm.)	
10	Yellowish brown, light yellow zones and colorless interzones; unidentified
14	Colorless interzone
5	Light yellow; all-*trans*-aurochrome (426, 401 mμ, in hexane)
52	Interzone
27	Light yellow; neoluteochrome-V (444, 417 mμ)
27	Interzone (contains traces of neoluteochrome-U)
119	Yellow; unchanged all-*trans*-luteochrome (449, 422 mμ)

3	Interzone
15	Light yellow ; neoluteochrome-A (443, 416 mμ)
61	Interzone
39	Light yellow ; neoluteochrome-B (444, 417 mμ)

Each zone was cut out separately and the individual eluates were transferred with water into hexane, washed, dried, and estimated photometrically. After evaporation of the respective hexane solutions, only unchanged all-*trans* zone was crystallized from benzene-methanol (m.p. 177∼178°) and was identified with the authentic sample. No isomer was able to be crystallized in spite of every effort, but their solution yielded, upon iodine catalysis, the corresponding all-*trans* form which did not separate on the column from the authentic all-*trans* sample.

Upon similar treatment of 71 mg. of the substance in 100 cc. of hexane with a catalytic amount (0.1 mg.) of iodine (illuminated for 5 min.) and the mixture developed on a column (60×7.5 cm.) during 1.5 hr., a large amount of neoluteochrome-U was obtained which hardly appeared in the previous experiment just described above.

Width of zone (mm.)

23	Unidentified yellowish brown zone, ligth yellow zone containing traces of all-*trans*-aurochrome, and colorless interzone
275	Colorless interzone
72	Yellow; neoluteochrome-U (449, 422 mμ, in hexane)
13	Interzone
73	Yellow; unchanged all-*trans*-luteochrome (449, 422 mμ)
30	Interzone
5	Light yellow; neoluteochrome-B (444, 417 mμ)

Despite its very similar characteristics of the ultraviolet spectrum to that of all-*trans* compound, neo-U was easily differentiated from all-*trans* or neo-V zone when subjected to the mixed chromatogram tests with them, and all-*trans* form was regenerated from neo-U isomer upon iodine catalysis treatment.

b) Photochemical Isomerization in the Absence of Catalysis : Upon other photochemical treatments such as illumination and insolation (cf. Table I), only a small extent of *trans*-*cis* rearrangement as well as furanoid oxide formation was observed.

c) Thermal Isomerization; Melting of Crystals : Ten milligrams of the substance was fused (cf. Table I), cooled, extracted with 50 cc. of cold hexane, and developed on a column for about 1 hr.

Width of zone (mm.)

5	Unidentified yellowish brown and light yellow zones
232	Colorless interzone
26	Light yellow; neoluteochrome-V (444, 417 mμ, in hexane)
26	Interzone
31	Yellow; unchanged all-*trans*-luteochrome (449, 422 mμ)
8	Interzone
12	Light yellow; neoluteochrome-A (443, 416 mμ)
2	Interzone
18	Light yellow; neoluteochrome-B (444, 417 mμ)

It is interesting that a large amount of neo-B isomer was formed under such a drastic condition.

When the substance was subjected to another thermal isomerization method, viz., refluxing the hexane solution of the substance, a few *trans*-*cis* rearrangement as well as furanoid oxide formation was shown on the chromatogram (cf. Table I).

Summary

1. When submitted to some thermal and photochemical treatments, luteochrome, $C_{40}H_{56}O_2$, suffers partial *trans*—*cis* isomerization and rearrangement into aurochrome. By means of chromatography a quantitative study of the resulting complex mixture was possible.

2. All-*trans* luteochrome was more stable to thermal and photochemical treatments than all-*trans*-β-carotene mono- and di-epoxides. However, considerable amount of stereoisomerization occurred when subjected to some treatments such as illumination in the presence of iodine or melting of crystals.

3. Luteochrome yielded four kinds of *cis* forms whose spectral characteristics were studied and their tentative configurational assignments were made. Some of these isomers were adsorbed on a chromatographic column, above or below the corresponding all-*trans* form. None of the *cis* forms crystallized.

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