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39. Nobuo Suzuki : *cis*-Mutatochromes

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Mutatochrome, a widespread natural provitamin A, $C_{40}H_{56}O$, was first isolated from orange peel by Karrer and Jucker,[1] and was named citroxanthin by the same authors. Although the structure (I) (Fig. 1) was established for this substance,[2],[*2] and some experiments on its vitamin A activity[3] were conducted, little attention has been paid for every case from the stereochemical points of view. In connection with the elucidation of the structure of the parent β-carotene *cis*-isomers,[*3] stereochemical studies on some *cis*-β-

Table I. Composition of the Mixtures of *cis-trans* Isomers obtained from All-*trans* Mutatochrome[a] (%)

Treatment	Unchanged all-*trans* form	neo-U	neo-A	neo-B	neo-C	neo-D	Irreversible loss
I_2 in light[b]	85	2	12	1	0	0	(trace)
Refluxing[c]	100	(trace)	(trace)	0	0	0	0
Illumination[d]	100	〃	〃	0	0	0	0
Insolation[e]	93	〃	7	0	0	0	(trace)
Melting crystals[f]	83	4	11	1	1	(trace)	1
Contacting with acid[g]	100	(trace)	(trace)	0	0	0	0

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 1% I_2 (pigment, 100%) was exposed to the light from a mercury lamp (Shimadzu 300-W) of 12 cm. in length without any light filter, from a distance of 24 cm. for 30 min.
c) The solution was refluxed in darkness for 1 hr.
d) 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.
e) Exposed to the direct sunlight of March for 2 hr.
f) The substance, in a thin-walled, evacuated tube, was immersed in a bath of 180° for 90 sec., then cooled in ice water.
g) *N* HCl (10 cc.) was added to hexane solution of the substance (1.5 mg. in 50 cc.) and the mixture was shaken for 30 min.

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*2 Recently, paprika pigments, capsanthin, capsorubin, and cryptocapsin were found to possess nonaëne-dione or decaën-one structure with cyclopentane end group(s) (R. Entschel, P. Karrer: Helv. Chim. Acta, **43**, 89 (1960); M. S. Barber, L. M. Jackman, C. K. Warren, B. C. L. Weedon: Proc. Chem. Soc., **1960**, 19), while Karrer's formula for general carotenoid furanoid oxides are still undisputable from their spectral data.
*3 Zechmeister, *et al.* assigned the 9-mono-*cis* and 9,13'-di-*cis* structures for neo-β-carotene-U and -B, respectively.

1) P. Karrer, E. Jucker : Helv. Chim. Acta, **27**, 1695 (1944).
2) *Idem* : *Ibid.*, **30**, 536 (1947).
3) N. T. Gridgeman, R. F. Hunter, N. E. Williams : J. Chem. Soc., **1947**, 131.

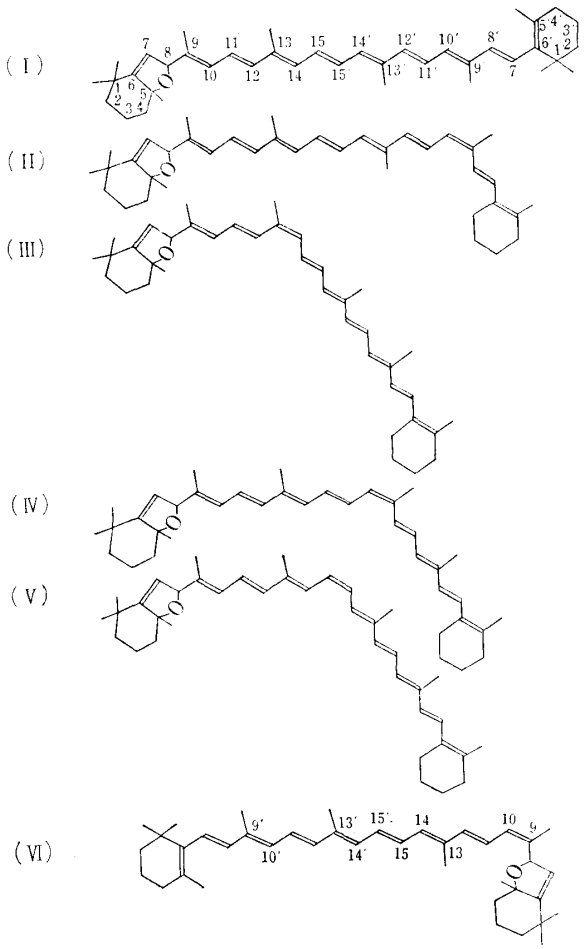

Fig. 1.

Carbon Skeleton Models of All-*trans* and Mono-*cis* Mutatochromes

carotene monoepoxides, diepoxides, and luteochromes (monoepoxide monofuranoid oxides) were previously reported by the present author in collaboration with Tsukida and Zechmeister.[4,5] In the current paper a quantitative and configurational studies on the second β-carotene furanoid oxide, mutatochrome, are presented.

All-*trans* mutatochrome (I) was converted into the expected stereoisomeric equilibrium mixtures after being treated by the usual methods to cause *trans*→*cis* isomerization of polyenes,[6] such as refluxing, illumination, insolation, contacting with acids, iodine catalysis of a solution, and melting crystals. Subsequent chromatographic resolution showed the presence of five *cis*-mutatochromes, listed in Table I, on the column. From each *cis* isomer all-*trans* compound was regenerated with iodine catalysis and was identified 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* mutatochrome was found to be more stable to light and heat than β-carotene mono- and di-epoxides and rather than luteochrome, though *trans*→*cis* stereoisomerization was still observed in this mutatochrome, especially when submitted to some drastic treatments such as iodine catalysis in light or melting crystals.

In contrast to the case of luteochrome previously reported,[5] illumination in the presence of iodine catalysis for shorter time did not give a peculiar neo isomer which showed no shift in its ultraviolet spectrum from that of all-*trans* compound. Furthermore, all-

4) K. Tsukida, L. Zechmeister : Arch. Biochem. Biophys., **74**, 408 (1958).
5) N. Suzuki, K. Tsukida : This Bulletin, **7**, 878 (1959).
6) L. Zechmeister : Experientia, **10**, 1 (1954); Chem. Revs., **34**, 267 (1944); Fortschr. Chem. org. Naturstoffe, **15**, 32 (1958).

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

Member of the set	Location of maxima in hexane ($m\mu$)[a]				$E^{mole}_{1cm.} \times 10^{-4}$	
					at λ_{max}	at *cis*-peak
Neo-mutatochrome-U	449,	*423*,	400(s),[b]	316	9.0	1.0
all-*trans*	454,	*425.5*,	405(s),	316	12.5	0.9
Neo-mutatochrome-A	449,	*423*,	400(s),	315	8.3	2.5
〃 -B	449,	*424*,	401(s),	315	5.9	3.0
〃 -C	442,	*417*,	395(s),	316	5.8	1.3
〃 -D	400～412				4.1	
Iodine equilibrium mixture	451,	*425*,	404(s),	316	5.5	1.7

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

a) The highest extinction observed at the maximum wave-length is given in italic letters.

b) shoulder.

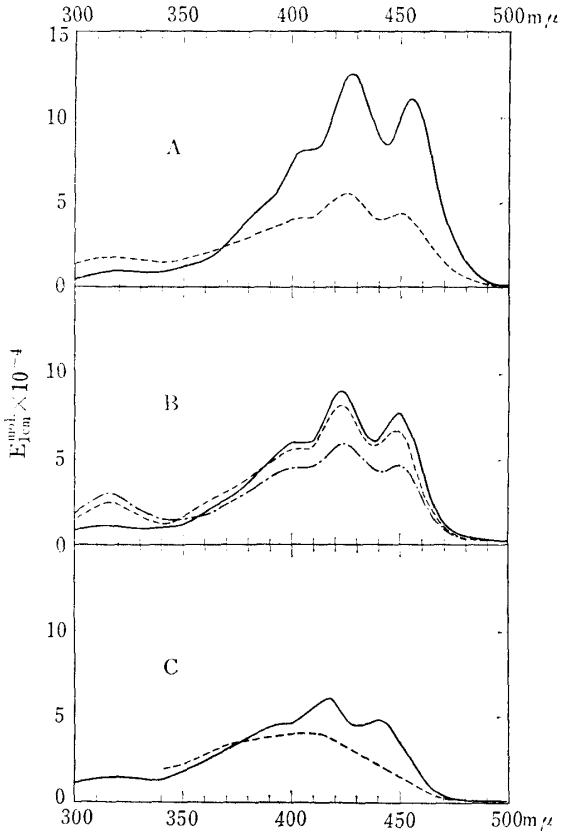

Fig. 2

Molecular Extinction Curves of Mutatochrome Set in Hexane

A: all-*trans* mutatochrome
——— fresh solution
------ mixture of stereoisomers after iodine catalysis
B: ——— neo-mutatochrome-U
------ 〃 -A
– – – – 〃 -B
C: ——— neo-mutatochrome-C
------ 〃 -D

trans compound was found to be very stable to dilute hydrochloric acid but was decomposed almost completely with concentrated acid without formation of any isomer of this set.

The spectral characteristics of five stereoisomers observed in the present study are listed in Table II. The formation of neo-mutatochrome-U (II), -A (III), and -B (IV) caused a shift of absorption maximum toward shorter wave-lengths by only 4.5, 4.5, and 3.5 mμ, respectively (Fig. 2, Table II). Since, according to earlier observations,[6,7] one *trans*→*cis*

7) 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); G. Karmakar, L. Zechmeister: *Ibid.*, **77**, 55 (1955); F. J. Petracek, L. Zechmeister: *Ibid.*, **78**, 1427 (1956); E. F. Magoon, L. Zechmeister: Arch. Biochem. Biophys., **69**, 535 (1957); W. V. Bush, L. Zechmeister: J. Am. Chem. Soc., **80**, 2991 (1958), etc.

rearrangement shifts the absorption maximum (in hexane) by 3 to 6 mμ toward shorter wave-lengths, these three isomers are assumed to be mono-*cis* compounds. The minor isomers, neo-C and neo-D, both obtained only in the case of melting crystals of all-*trans* compound, have at least two *cis* bonds and they may aptly be represented as a di-*cis* and a tri- or tetra-*cis* isomers, respectively. It is not surprising that neo-D compound lacks a fine structure in its spectrum because of its poly-*cis* character.[6,7] These two isomers were not studied further at present time.

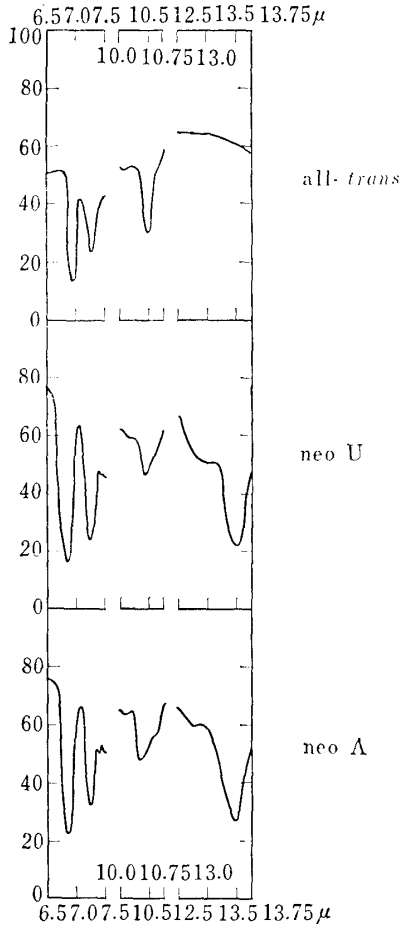

Fig. 3.
Infrared Spectra of Mutatochrome Set
All-*trans*: Nujol mull
Neo isomers: ca. 0.01 mm. liquid

In Fig. 3, the infrared spectra of all-*trans*, neo-U, and neo-A forms are given and these show a characteristic band at 7.27, 7.24, and 7.23 μ, respectively, which may be interpreted as the deformation vibrations of methyl group or typical methylated *cis*-double bond in polyenes.[8,9] It is also pointed out that these peaks of the above *cis*-isomers are located almost at the same wave-lengths and are in a shorter region than that of all-*trans* form.

It was emphasized by Lunde and Zechmeister[9] that *cis*-carotenoids which have methylated *cis*-double bond(s) exhibits a typical band at exactly 7.25 μ while this is missing in the corresponding parent all-*trans* form. However, result of studies on β-carotene mono- and di-epoxides,[4] luteochrome,[5] and the present substance is not coincident with their conclusion.

The band at 10.45 μ is known to be the out-of-plane vibration of the *trans*-double bond. All-*trans* form shows such a band at 10.45 μ while neo-U and neo-A give a similar but weaker band than that of all-*trans* form at 10.43 and 10.39 μ, respectively. It has been

8) R. S. Rasmussen, R. R. Brattain: J. Chem. Phys., **15**, 120 (1947).
9) K. Lunde, L. Zechmeister: Acta Chem. Scand., **8**, 1421 (1954); J. Am. Chem. Soc., **77**, 1647 (1955).

noted[9] that stereoisomers containing unmethylated *cis*-double bond(s) exhibit a characteristic strong band at 12.84~12.94 μ and splitting of the band at 10.3~10.5 μ into doublet or triplet should also appear. Since these peaks are clearly missing in the spectra of neo-U and neo-A forms, the 15,15'-*cis* configuration (V) for neo-U and neo-A might be excluded. 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.

Since a sufficient amount of neo-B was not available, infrared spectral measurement of this compound had to be abandoned, but 15,15'-*cis* configuration (V) might also be excluded for this isomer from the evidence of its similarity to the corresponding *cis*-luteochrome[5] and from the fact that no central *cis*-isomer has ever been formed from all-*trans* form *in vitro* by usual isomerization method in all of carotenoid pigments studied so far.[6] Considering the weak shift of the absorption maximum, high *cis* peak, and low molecular extinction values in its ultraviolet spectrum, this neo-B isomer should be mono-*cis* compound, the *cis*-double bond of which would be located nearer the center of the chromophore than that in neo-A form.

Mutatochrome has 10 double bonds, eight of which can take part in *trans*→*cis* isomerization. However, three *cis*-isomers obtained in the present experiment exhibited comparatively high extinction values and characteristic fine structures in definition of their ultraviolet spectra, and neither ultraviolet nor infrared spectral characteristics showed any unmethylated-*cis* formation around the sterically hindered carbon bond, viz., 11-12, 11'-12', or 7'-8' bond. Consequently, either one of left four bonds, at 9-10, 13-14, 13'-14', and 9'-10', may be available for the present mono-*cis* isomerization. Furthermore, 9-10 mono-*cis* form (VI), which has a *cis*-configuration around the double bond nearest the furan ring, should exhibit no shift from the parent all-*trans* compound in its ultraviolet spectrum, as pointed out in the case of *cis*-aurochrome[4] and neo-luteochrome-U.[5] Since such a noteworthy feature is missing in either neo-mutatochrome-A, -B, or -U, *cis*-isomerization may not appear around 9-10 position in the present stereoisomeric set. Considering the nonsymmetrical molecular shape of mutatochrome, strength of the *cis*-peak should be in the order of 13'-14'>13-14>9'-10'. On the basis of molecular extinction coefficients at their maximum wave-lengths and the strength of *cis* peaks described above, the overall molecular shape of neo-A or neo-B has to be a bent one and its *cis* bond would be located near the center of the chromophore, while neo-U has an essentially straight molecular shape containing a peripheral *cis*-bond. From all the available experimental data and the previous studies, a tentative proposal is presented that neo-mutatochrome-U has the 9'-10' mono-*cis* (II), neo-A the 13-14 mono-*cis* (III), and neo-B the 13'-14' mono-*cis* (IV) configurations.

Experimental

Materials and Methods——For the present purpose, all operations were carried out in dim light and the evaporation was made *in vacuo* at around room temperature. For washing of solutions, the LeRosen automatic apparatus[10] was used. Melting points were measured in an evacuated capillary, in an electrically heated Berl block, and corrected. In chromatography, $Ca(OH)_2$ (Wako Pure Chem. Ind., Ltd.) was found to be the best adsorbent. Hexane-Me_2CO mixture (98:2) and pure Me_2CO were used as the developer and eluant, respectively. 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 was 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

10) A. L. LeRosen: Ind. Eng. Chem., Anal. Ed., **14**, 165 (1942).

set. Further, 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 recording spectrophotometer EPS-2 was used.

Main source for the sample of all-*trans* mutachrome 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] Mutatochrome was also obtained from β-carotene monoepoxide by using the usual isomerization technique as small leaf-shaped orange crystals (from benzene-MeOH), m.p. 165~166°. *Anal.* Calcd. for $C_{40}H_{56}O$: C, 86.89; H, 10.21. Found : C, 86.34; H, 10.18. UV λ_{max}^{hexane} mμ ($E_{1cm}^{mole} \times 10^{-4}$) : 454(11.2), 427.5(12.5), 405(8.2), 316(0.9).

Isomerization of All-*trans* Mutatochrome (cf. Table I)——a) Iodine catalysis, in light : A solution containing 3 mg. of a substance and 0.03 mg. of I_2 in 50 cc. of hexane was illuminated for 30 min. and then developed on a chromatographic column for 90 min. The following chromatogram was obtained :

Width of zone (mm.)	
3	Colorless zone
3	Light yellow; unidentified
21	Colorless interzone
2	Light pink; unidentified
84	Interzone
16	Light yellow; neo-mutatochrome-U (449, 423 mμ, in hexane)
2	Interzone
67	Yellow; all-*trans* mutatochrome (454, 427.5 mμ)
52	Interzone
31	Light yellow; neo-mutatochrome-A (449, 423 mμ)
15	Interzone
13	Light yellow; neo-mutatochrome-B (449, 424 mμ)

Each zone was cut out separately and the individual eluates were transferred with H_2O into hexane, washed, dried, and estimated photometrically. After evaporation of the respective hexane solutions, only unchanged all-*trans* zone crystallized from benzene-MeOH (m.p. 165~166°) and identified with the authentic sample. None of the *cis*-isomers 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.

b) Photochemical isomerization in the absence of catalyst : Upon other photochemical treatments such as illumination and insolation (cf. Table I), only a trace or a small extent of *trans*→*cis* rearrangement was observed.

c) Thermal isomerization; melting of crystals : Four mg. of all-*trans* compound was fused (cf. Table I), cooled, extracted with 50 cc. of cold hexane, and developed on a column for about 90 min.

Width of zone (mm.)	
4	Light yellow; unidentified
16	Colorless interzone
3	Light pink; unidentified
86	Interzone
24	Light yellow; neo-mutatochrome-U (449, 423 mμ, in hexane)
2	Interzone
69	Yellow; all-*trans* mutatochrome (454, 427.5 mμ)
27	Interzone
29	Light yellow; neo-mutatochrome-A (449, 423 mμ)
16	Interzone
15	Light yellow; neo-mutatochrome-B (449, 424 mμ)
23	Interzone
18	Light yellow; neo-mutatochrome-C (442, 417 mμ)

After being concentrated *in vacuo* and rechromatographed, the light yellowish filtrate gave neo-mutatochrome-D (400~412 mμ in hexane).

When the all-*trans* compound was subjected to another thermal isomerization by refluxing its hexane solution, only a trace of *trans*→*cis* rearrangement was observed on the chromatogram (cf. Table I).

d) Contacting with acid : When the all-*trans* compound was treated with *N* HCl, only a trace of *trans*→*cis* rearrangement was found on the chromatogram (cf. Table I), while this was almost entirely decomposed by contacting with conc. HCl.

The author is grateful to Professors Z. Horii and K. Tsukida for their encouragements and valuable discussions throughout this investigation. He is indebted to Mr. Nakamachi of Takeda Chemical Industries for the infrared spectra.

Summary

1) All-*trans* mutatochrome, $C_{40}H_{56}O$, undergoes rearrangement to give *cis* isomers which can be resolved by column chromatography.

2) Five *cis* compounds were isolated, of which three are mono-*cis* isomers, termed neo-U, -A, and -B.

3) Quantitative study of the stereoisomeric mutatochrome was made and all-*trans* mutatochrome was found to be more stable to thermal, photochemical, and catalytic treatments than all-*trans* luteochrome.

4) Some related spectroscopic phenomena were discussed and tentative configurational assignments of (II), (III), and (IV) were respectively proposed for neo-U, -A, and -B.

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40. Tsutomu Momose and Akira Inaba : Organic Analysis. XXVIII.[1] Mechanism of the Color Reaction of 3,6-Dinitrophthalic Acid with Reducing Sugars.

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3,6-Dinitrophthalic acid gives a very sensitive color reaction with a reducing sugar and is successfully used in the microdetection,[2] approximate estimation,[3] and determination[1,4] of the sugar. When the reagent dissolved in aqueous alkaline solution is heated with a small amount of a reducing sugar, a deep wine-red color appears in a few minutes. This initial color is unstable but, if the reaction is carried out for a longer time (10 minutes) in the presence of sodium thiosulfate, a very stable orange-red color is produced. This paper presents the mechanism of these reactions, isolating the main coloring matters in a crystalline form.

Coloring Matter of the Initial Unstable Color Reaction

The initial wine-red color of the reagent produced with glucose in the absence of sodium thiosulfate fades gradually to a faint yellow from the upper part of the colored solution, where the coloring matter is in contact with the air. The absorption spectrum of the color could only be observed by adding sodium metaphosphate in the solution and such a spectrum is shown in Fig. 1-A.

*1 Katakasu, Fukuoka (百瀬 勉, 稲葉 頴).

1) Part XXVII : T. Momose, Y. Mukai : Yakugaku Zasshi, **81**, 227 (1961).
2) T. Momose, A. Inaba : This Bulletin, **7**, 541 (1959).
3) T. Momose, A. Inaba, Y. Mukai, T. Shinkai : *Ibid.*, **8**, 514 (1960).
4) T. Momose, Y. Mukai, M. Watanabe : Talanta, **5**, 275 (1961).