Phytochemistry, 1972, Vol 11, pp 401 to 404 Pergamon Press Printed in England

SHORT COMMUNICATION

MIMULAXANTHIN—A NEW ALLENIC XANTHOPHYLL FROM THE PETALS OF *MIMULUS GUTTATUS*

Helfried Nitsche
Botanisches Institut der RWTH, 51 Aachen, Deutschland

(*Received* 11 *February* 1970)

Abstract—Besides neoxanthin and deepoxineoxanthin, the main xanthophylls in the petals of *Mimulus guttatus*, an additional pigment, mimulaxanthin, has been obtained, it is 3,3′-5,5′-tetrahydroxy-6′-hydro-7-dehydro-β-carotene Its neoxanthin-like spectrum is not changed by acids Two hydroxyl groups are acetylatable The diacetate can be silylated to a di(trimethylsilyl)-ether, and with acidic alcohols, yields a monoether With $CHCl_3$ plus HCl 18 defined reaction-products are obtained, one of which is 3,3′-di-hydroxy-7,8-didehydro-α-carotene (monadoxanthin) One of the $LiAlH_4$-reduction products of neoxanthin is identical with mimulaxanthin, whereas mimulaxanthin with $LiAlH_4$ gives an acetylenic derivative These results have been confirmed by IR spectrometry Mimulaxanthin is not an artefact formed during extraction and purification

INTRODUCTION

As in many other petals, the xanthophylls of *Mimulus guttatus* are esterified with fatty acids. These esters can be analysed by fractionation on columns of silica gel and TLC on cellulose partly impregnated with paraffin-oil.[1] After saponification the free pigments are obtained. Fractionating saponified extracts from petals of *M. cupreus* and *M tigrinus* on columns with $ZnCO_3$–celite, Goodwin and Thomas[2] found very strongly adsorbed xanthophylls, which they characterized as follows. Pigment B resembled zeaxanthin in its spectral properties, but was more strongly adsorbed than authentic material Pigment C had the same spectrum as taraxanthin from *Taraxacum officinale* and did not separate from it on co-chromatography Pigment D was still more strongly adsorbed than pigment C, but had the same spectrum. Because of difficulties in handling such strong adsorbed xanthophylls, they were not further identified In earlier publications it has been shown that pigment B is probably identical with deepoxineoxanthin and pigment C with neoxanthin.[3,4] In this paper, the identity of pigment D with mimulaxanthin is discussed

RESULTS AND DISCUSSION

TLC of the saponified extract from *M guttatus* petals on partly impregnated cellulose separates the xanthophylls present. In this system, deepoxineoxanthin (3 OH, 1 allene[4]), which probably is identical with pigment B,[2] has nearly the same R_f as violaxanthin (2 OH, 2 epoxides) Mimulaxanthin has a higher R_f than neoxanthin (3 OH, 1 epoxide, 1 allene[5]), which is probably the same as pigment C,[2] but a lower one than vaucheriaxanthin (4 OH, 1 epoxide, 1 allene[6]) So its R_f is in accordance with 4 OH or 3 OH plus 2 O. On columns of

---

[1] H Kleinig and H Nitsche, *Phytochem* **7**, 1171 (1968)

[2] T Goodwin and D Thomas, *Phytochem* **3**, 47 (1964)

[3] K Egger, A Dabbagh and H Nitsche, *Tetrahedron Letters* **35**, 2995 (1969)

[4] H Nitsche, K Egger and A Dabbagh, *Tetrahedron Letters* **35**, 2999 (1969)

[5] L Cholnoky, A Ronai, J Szabolcs, E Nagy, C Toth, G Galasko, A Mallams, E Waight and B Weedon, *J Chem Soc* 1256 (1969)

[6] H Nitsche and K Egger, *Tetrahedron Letters* 1435 (1970)

basic $ZnCO_3$ or TL of basic $MgCO_3$[7,8] this pigment is adsorbed very strongly, it differs in behaviour from authentic xanthophyll markers

The functional groups were determined as follows 0 01 N HCl in EtOH shifts the maxima of the neoxanthin-like spectrum only slightly towards shorter wavelengths as in *trans–cis* isomerization, indicating the absence of rearrangeable epoxide groups Isolated epoxide groups as in fucoxanthin[9] can be eliminated by $LiAlH_4$, as has been shown for *Vaucheria* heteroxanthin,[10] no such elimination was achieved with mimulaxanthin $NaBH_4$ (EtOH, 40°) has no effect on spectrum or R_f so that keto groups are not present in the molecule. Formation of a diacetate indicates 2 primary or secondary hydroxyls The

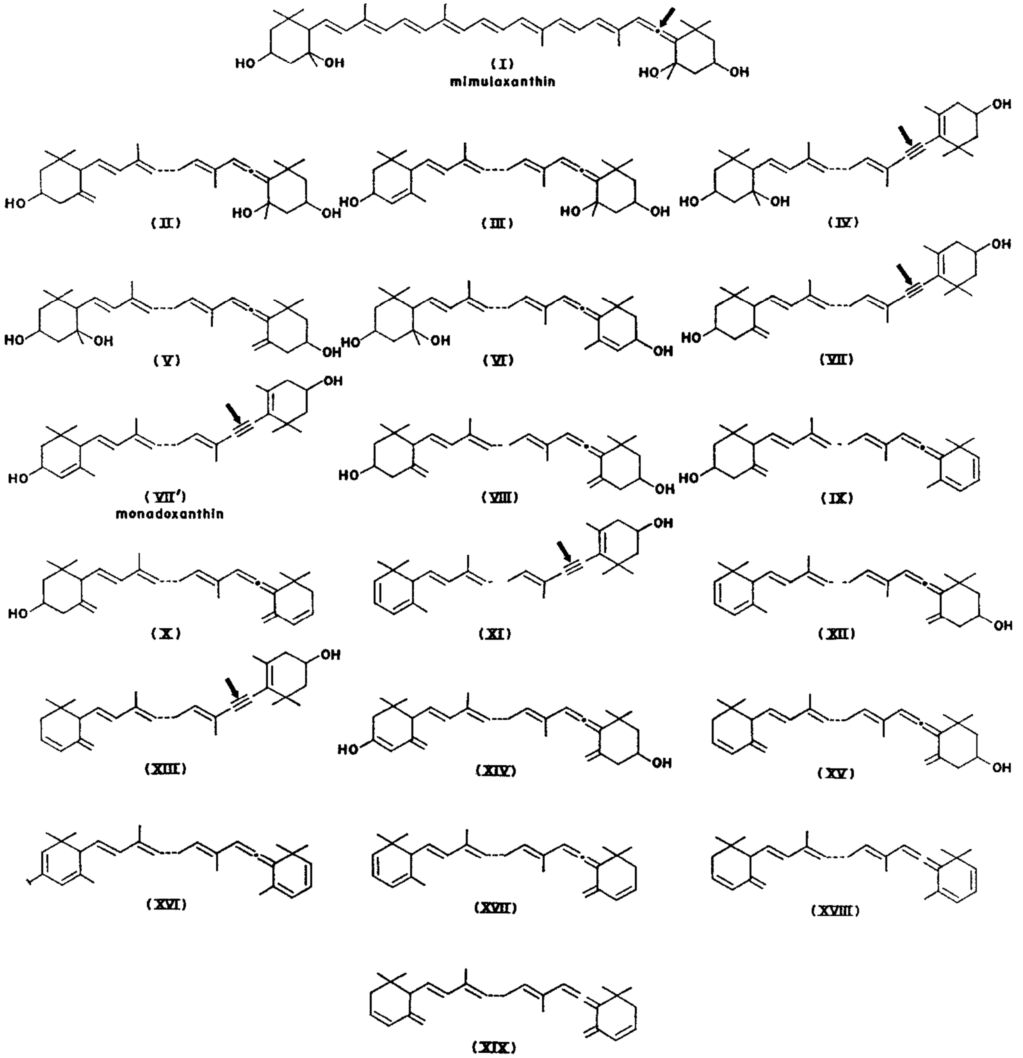

FIG 1 MAIN REACTION PRODUCTS OF MIMULAXANTHIN WITH ACIDIC $CHCl_3$

---

[7] K EGGER, *Planta* **80**, 65 (1968)

[8] H NITSCHE and K EGGER, *Phytochem* **8**, 1577 (1969)

[9] R BONNETT, A MALLAMS, T TEE and B WEEDON, *Chem Commun* 515 (1966)

[10] H NITSCHE, *Tetrahedron Letters* 3345 (1970)

diacetate can be silylated to a di(trimethyl-silyl)-ether, showing the presence of two tertiary OH. Treatment of mimulaxanthin-diacetate with acidic alcohols yields 1 monoether, indicating one of the tertiary OH to be allylic.[11] The IR spectrum of mimulaxanthin (KBr, Leitz III G) exhibited (cm^{-1}): 3500 (OH), 3040, 2965, 2865 (CH_2, CH_3), 1990, 1950 (terminal allene), 1570, 1550 (conj. C=C), 1480, 1460 (CH_2), 1390, 1380, 1355 (CH_3, gem. CH_3), 1310 (tert OH), 1245, 1210 (*trans* di-subst C=C), 1170, 1145 (tert OH), 1110 (allylic OH), 1070 (allene), 1040, 1020 (sec. OH or allylic prim OH), 1005, 975, 953 (*trans* di-subst. C=C) There were no maxima for $C\equiv C$ (2150), C=O (1710), conj C=O (1640), epoxide (1250, 900, 800) or $—OCH_3$ (2820)

Hence, mimulaxanthin has two secondary and two tertiary hydroxyl groups, one of which is allylic, and an allene group Since there was not enough pigment for NMR and MS, the positions of the 4 OH and the allene were determined by other means With acidic $CHCl_3$ (0·01 N HCl, 20°, 10′(!)), a reagent which specifically eliminates *tert.* OH and *sec.* allylic OH,[3,4,11–15] 18 reaction products were obtained (Fig 1). In this scheme the intermediary products, which continue reacting, are not depicted (except monadoxanthin VII′, its isomer VII and the trihydroxy derivatives) They will be discussed in detail elsewhere Here the properties of monadoxanthin (VII′) are described. This pigment was first isolated from flagellates of the algal class Cryptophyceae[16] and its examination has revealed the existence of an acetylenic bond[17] as it was proved for diatoxanthin, diadinoxanthin, alloxanthin and crocoxanthin [17,18] The pigment derived from mimulaxanthin, which is only obtained after a very short reaction time (5 min), can be separated from the other products by TLC on $MgCO_3$ [7,8] It absorbs at slightly longer wavelengths than lutein (*trans*· 476, 448, 425 nm, EtOH), yields a diacetate and a monoether The R_fs differ from lutein in the same way as those for zeaxanthin and diatoxanthin differ from antheraxanthin and diadinoxanthin [18] The IR spectrum exhibited (cm^{-1}, KBr)· 3380 (assoc OH), 3040, 2965, 2865 (CH_2, CH_3), 2160 ($C\equiv C$), 1570, 1550 (conj C=C), 1480, 1460 (CH_2), 1390, 1380 (CH_3, gem CH_3), 1245, 1210 (*trans* disubst, C=C), 1110 (allylic OH), 1040, 1020 (sec. OH or allylic prim OH) The transformation of the allenic into the acetylenic bond can be understood as an attack of H^+ at the tert OH of $C_{5'}$ and stabilization of the intermediary carbonium-ion by elimination of a proton at $C_{8'}$ (Fig 2) By this reaction mechanism, neoxanthin can be transformed into diadinochrome, and deepoxineoxanthin into diatoxanthin [3,4] Since mimula xanthin resembles neoxanthin, it might be expected to be

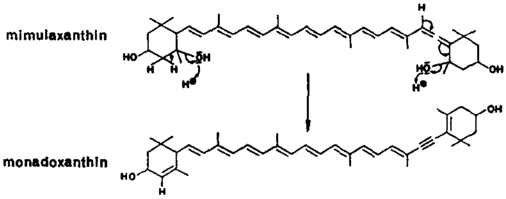

FIG 2 TRANSFORMATION OF MIMULAXANTHIN INTO MONADOXANTHIN WITH ACIDIC $CHCl_3$.

---

[11] E GROB, *Angew Chem* **16**, 784 (1966)

[12] E GROB and R PFLUGSHAUPT, *Helv Chim Acta* **45**, 1592 (1962)

[13] E GROB and R PFLUGSHAUPT, *Helv Chim Acta* **48**, 930 (1965)

[14] B SCHIMMER and N KRINSKY, *Biochemistry* **5**, 3649 (1966)

[15] A HAGER and H STRANSKY, *Arch Mikrobiol* **71**, 132 (1970)

[16] D CHAPMAN, *Phytochem* **5**, 1331 (1966)

[17] A MALLAMS, E WAIGHT, B WEEDON, D CHAPMAN, F HAXO, T GOODWIN and D THOMAS, *Chem. Commun* 301 (1967)

[18] K EGGER, H NITSCHE and H. KLEINIG, *Phytochem* **8**, 1583 (1969)

formed on reduction of neoxanthin with $LiAlH_4$ Chromatographic separation of the reaction products either by TLC on cellulose partly impregnated with plant-oils or on $MgCO_3$ yielded 4 pigments, one of which was identical with mimulaxanthin (Fig 3) It gave diacetates, diacetate–disilylethers, diacetate–monoethers, and with $CHCl_3$ plus HCl the already mentioned derivatives. The IR spectrum showed that the allene group was intact Deepoxineoxanthin, the second product of reduction, was transformed into diatoxanthin with acidic $CHCl_3$[4] On the other hand, diatoxanthin is also obtainable by reduction of neoxanthin (and deepoxineoxanthin) with $LiAlH_4$, probably by an attack of H^- on the tert. OH (Fig 3)[19] The second acetylenic derivative of neoxanthin is hydroxy-diatoxanthin This pigment can also be obtained from mimulaxanthin (Fig 3) Reducing foliachrome= neochrome=furanoic neoxanthin with $LiAlH_4$, other workers obtained zeaxanthin[20–22]

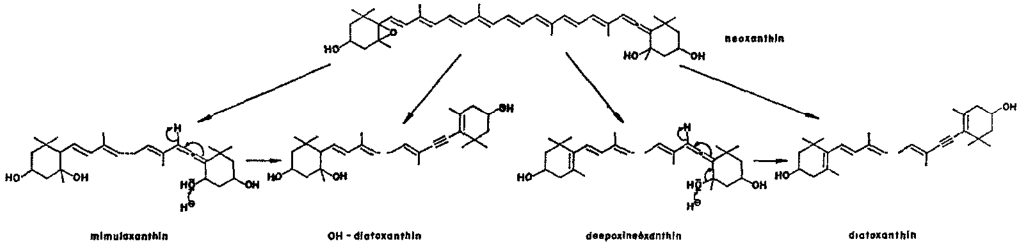

FIG 3 TRANSFORMATION OF NEOXANTHIN INTO MIMULAXANTHIN WITH $LiAlH_4$

In order to see whether mimulaxanthin is an artefact produced during extraction or saponification, from neoxanthin, this xanthophyll (and deepoxineoxanthin) was treated with excess Na alcoholate (20%, 12 hr, 40°) No traces of mimulaxanthin (or other products) could be obtained

EXPERIMENTAL

Isolation After addition of basic $MgCO_3$ freshly harvested or frozen petals of *M guttatus* (Botanischer Garten Heidelberg resp Aachen) were ground in a mortar, extracted with acetone and the xanthophyll ground esters transferred to light petroleum They were saponified with Na alcoholate (0 5%) or CH_3OH plus NaOH (3%) at room temp (4 hr) Na salts of the fatty acids were washed out with water after addition of Et_2O, the free xanthophylls evaporated to dryness and redissolved in Et_2O–light petroleum (1 25)

Chromatography Partition TLC[23] 20 g cellulose (MN 300) are dissolved in dioxane (70 ml) and then H_2O (70 ml) is added 4 glass plates (20 × 20 cm) are coated with the pulp, dried at room temp, impregnated with sunflower-resp paraffin-oil (8% resp 10% in petroleum b p 100–120°) leaving a margin (2 cm) unimpregnated Here the pigments are put on and developed with $MeOH$-Me_2CO-H_2O (40 10 3), resp Me_2CO–MeOH Adsorption TLC[7,8] 20 g $MgCO_3$ (basic, Merck 5828) + 10 g kieselguhr (Merck 8117) are suspended in 250 ml acetone, shaken, spread over glass plates, dried (50°) and developed with light petroleum–benzene–Me_2CO *Column chromatography* The xanthophylls are separated in columns (5 × 10 cm) on $ZnCO_3$ (basic, Merck 5661) with light petroleum–Me_2CO

Derivatives Acetylation Pigments were dissolved in dry pyridine (2 ml), Ac_2O (0 5 ml) was added and the reaction mixture allowed to stand at 40° *Silylation* To the pigment in dry pyridine (2 ml) 3 drops of $(CH_3)_3$ SiCl are added After 1 hr at room temp ether is added and washed with water *$LiAlH_4$ reduction* To the pigment in dry ether 0 5 g $LiAlH_4$ (Merck 5661) in crude form are added (inverse addition) and shaken vigorously (1 hr, 20°) The complex is destroyed and the strongly adsorbed reaction—products are transferred into ether by elution with methanol, dried, evaporated, and dissolved in Et_2O–light petroleum (1 25)

Dehydration The pigments react in 0 01 N HCl-acidic $CHCl_3$

Acknowledgement—I am indebted to Miss H Corr for operating the IR spectrometer

---

[19] H NITSCHE, *Tetrahedron Letters* 3343 (1970)

[20] R. BONNET, A SPARK, T TEE and B WEEDON, *Proc Chem Soc* 419 (1965)

[21] B WEEDON, *Chem in Brit* 3, 428 (1967)

[22] L CHOLNOKY, K GYORGYFY, J SZABOLCS, B WEEDON and E WAIGHT, *Chem Commun* 404 (1966)

[23] K EGGER, *Chromatography Symp II*, 75, Brussel (1962)

Key Word Index—Scrophulariaceae, mimulaxanthin, allenic xanthophyll, *Mimulus guttatus*, yellow flower pigment