EXPERIMENTELLES

NMR. Bruker WH 270, TMS als innerer Standard; **IR**: Beckman IR 9, in CCl_4; MS: Varian MAT 711 mit Datenverarbeitung, 70 eV. 95g zerkleinerte Wurzeln (lufttrocken) extrahierte man mit Et_2O–Petroläther (=PÄ) (1 : 2) und trennte den erhaltenen Extrakt durch mehrfache PLC (Si gel, GF 254, 1mm), Et_2O/PÄ 1 : 3 bzw. 1 : 1. Man erhielt 4 mg **1** und 3 mg **2** und **4**, die 1 hr mit Acetanhydrid auf 70° erwärmt wurden. Nach Eindampfen i. Vak. erhielt man nach PLC (Et_2O–PÄ 1 : 1) *ca* 1mg **3** und 1 mg **4**.

8-*Oxo-β-cyperon* (**1**). Nach Destillation i. Vak. (Sp. 90–100° 0.1 Torr) erhielt man farblose Kristalle aus Petroläther, Schmp. 85°. IR: 1687, 1670, 1600 cm^{-1} (C=C C=O). UV: $\lambda_{max} = 304$ nm ($\epsilon = 17000$). MS: M^+ *m/e* 232.146 (100%) (ber für $C_{15}H_{20}O_2$ 232.146); –Me 217 (31).

$$\frac{\lambda \quad 589 \quad 578 \quad 546 \quad 436\text{nm}}{[\alpha]_{24} \quad +404 \quad +429 \quad +525 \quad +1645} \ (c = 0.27)$$

2*β-Acetoxy-8-oxo-β-cyperon* (**3**): Farbloses Öl, IR: 1750, 1230 (OAc); 1680, 1600 (C=C CO)cm^{-1}. MS: M^+ *m/e* 290.152 (12%) (ber. für $C_{17}H_{22}O_4$ 290.152); –$H_2C{=}C{=}O$ 248 (24); –AcOH 230 (9) 248 –Me 233 (11); 230 –CO 202 (100).

11-*Hydroxy-8-oxo-β-cyperon* (**4**). Farbloses Öl, IR: 3620 (OH); 1670, 1600 (C=C–CO)cm^{-1}. MS: M^+ *m/e* 248.141 (4%) (ber. für $C_{15}H_{20}O_3$ 248.141): –Me 233 (100); 233 –H_2O 205 (57).

Anerkennungen—Der Deutschen Forschungsgemeinschaft danken wir für die Förderung dieser Arbeit, insbesondere für das 270 MHz-Gerät.

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ISOLATION OF FREE *CIS* AND *TRANS*-PHYTOL FROM THE RED ALGA *GRACILARIA ANDERSONIANA*

JAMES J. SIMS and JOHN A. PETTUS, JR.
Department of Plant Pathology and Chemistry, University of California, Riverside, CA 92502, U.S.A.

(*Received* 8 *October* 1975)

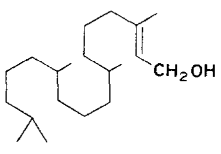
Key Word Index—*Gracilaria andersoniana*; Gracilariaceae; alga; free phytols.

The diterpene alcohol phytol is unusual among terpenes in that it has a definite role in plants as part of the chlorophyll molecule. Perhaps because of this important function in photosynthesis, there apparently never has been a report of free phytol and only one report of an isomer [1] existing in a plant. In fact there is evidence that phytol is not accumulated in etiolated leaves [2] and that phytol is involved in control of chlorophyll biosynthesis [3] in *Phaseolus vulgaris*.

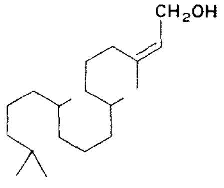
We have been investigating the secondary metabolites of a number of marine algae and note here our results on the red alga *Gracilaria andersoniana*. Hot hexane extraction of the air dried, ground alga gave a green oil which was chromatographed on silica gel. The phytols were eluted in several fractions which were pooled and rechromatographed by high pressure liquid chromatography to yield pure *trans*-phytol **1** and *cis*-phytol **2**. Three other compounds isolated from the extract were cholesterol, oleic and linoleic acids.

Extraction of the plant material remaining after hexane extraction with CH_2Cl_2 in a Soxhlet yielded a dark green oil. Saponification of this extract according to the "normal" phytol isolation procedure [4] yielded only the natural *trans*-phytol. Both **1** and **2** were also found in a cold MeOH–$CHCl_3$ extract of the fresh, wet alga, and a cold hexane extract of the air dried alga.

The identification of **1** and **2** was based on the work of Burrell *et al.* [5], who synthesized both phytols, and comparison of the published [6] NMR spectrum of **1**. The significant spectroscopic differences between **1** and **2** are found in their NMR spectra. The 3-methyl groups in **1** and **2** absorb as singlets at 1.64 and 1.71 compared to the reported values of 1.62 and 1.73 respectively [5]. The 1-methylene groups are found as doublets at δ 4.05 and δ 4.48 in **1** and **2** respectively, while the position of the olefinic proton in both compounds is δ 5.33. The downfield position of the 1-methylene in **2** with respect to **1** can be explained in terms of intramolecular van der Waals deshielding between the 1-methylene group and the moderately bulky C_{16}-group which are *cis*-substituted at the double bond.



(**1**)



(**2**)

This is the first isolation of *cis*-phytol from a natural source. It is only present in the free form. The phytol isolated from saponification of chlorophyll is the usual *trans* isomer. The presence of phytol in Chlorophyta [7], Phaeophyta [4] and Cyanophyta [4] has been reported. Now a representative of the Rhodophyta can be included in this group.

EXPERIMENTAL

Isolation. Graciliaria andersoniana collected intertidally at Laguna Niguel, California was air dried. The finely ground material (2.64 kg) was Soxhlet extracted with hexane for 48 hr. Removal of the hexane *in vacuo* yielded 5.33 g of a green oil (0.21%). This extract was chromatographed on an open Si gel column (145 g). With 5% EtOAc in hexane a fraction containing **2** eluted followed by a fraction containing **1**; cholesterol was eluted with 20% EtOAc in hexane and oleic and linoleic acids with 50% EtOAc in hexane. Crude *trans*-phytol was further purified using a Water Associates high pressure liquid chromatograph equipped with 244 × 0.94 cm Si gel column (Porasil A, 37–75 μm). *cis*-Phytol was also purified by HPLC (a 700 × 0.95 cm plus a 460 × 0.64 cm column). The solvent system used was 3% EtOAc in hexane.

trans-*Phytol* (**1**). Pure **1** was isolated as a clear colorless oil (0.014% yield from dry wt of alga). IR (KBr) 3.00, 6.82, 7.23 and 9.9 μm; NMR (CCl_4, δ), 0.90 (12H, *d*, *J* 6 Hz), 1.2–1.6 (20H, *m*), 1.64 (3H, *s*), 1.95 (2H, *m*), 4.05 (2H, *d*, *J* 6.4 Hz), 5.33, (1H, *t*, *J* 6.4 Hz); MS molecular ion *m/e* 296 (corresponds to $C_{20}H_{40}O$).

cis-*Phytol*. Compound **2** was isolated as a clear colorless oil (0.0028% yield from dry wt of alga). IR (CCl_4) 2.92, 6.86, 7.30, 10.4 μm; NMR (CCl_4, δ), 0.91 (12H, *d*, *J* 6 Hz), 1.2–1.6 (20H, *m*), 1.71 (3H, *s*), 2.0 (2H, *m*), 4.48 (2H, *d*, *J* 6.4 Hz), 5.33 (1H, *t*, *J* 6.4 Hz); MS molecular ion *m/e* 296 (corresponds to $C_{20}H_{40}O$).

Acknowledgement—This research was supported by a grant from the National Science Foundation. We are grateful to Glenn Van Blaricom for identification of the alga.

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THE ISOLATION OF γ-CAROTENE AND A POLY-*CIS*-γ-CAROTENE FROM THE TANGERINE TOMATO*

Richard W. Glass and Kenneth L. Simpson
Department of Food and Resource Chemistry, University of Rhode Island, Kingston, RI 02881, U.S.A.

(*Revised received* 24 *September* 1975)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tangerine tomato; poly-*cis*-γ-carotene; γ-carotene.

Plant and source. Lycopersicon esculentum; Tangerine tomato genotype, variety Golden Jubilee. Field-ripened Tangerine tomato fruits were used in this study. The seeds were a generous gift from Dr. M. L. Tomes of the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana, U.S.A.

Present work. Tangerine (Golden Jubilee) tomato fruits are characterized by high concentrations of the poly-*cis* carotenes, proneurosporene and prolycopene [1,2] and poly-*cis*-ζ-carotene [3]. In further investigations, a pink contaminant of proneurosporene was resolved into two bands: the upper from the MgO–Hyflo SuperCel column was identified as γ-carotene (1.8 μg/g dry w), and the lower as a poly-*cis*-γ-carotene (3.0 μg/g dry w). Poly-*cis* carotenes normally exhibit strong bathochromic shifts and an increase in extinction upon I_2 catalysis. The spectrum of poly-*cis*-γ-carotene exhibits two maxima which do not shift; however, a third absorption peak is formed and the spectrum was then identical to that of the stereoisomers obtained upon I_2 catalysis of γ-carotene extracted from carrots and high-beta tomatoes (Table 1). The wavelength maxima for both γ-carotene and poly-*cis*-γ-carotene are lower than the literature values [4–7], but the purified γ-carotene isolated from Tangerine tomatoes was identical to a sample isolated from Summer Sunrise or high-beta tomatoes and carrots.

No cyclic poly-*cis* carotenes have been reported previously. It remains to be seen if pro-γ-carotene from *Pyracantha angustifolia* is identical with this poly-*cis*-γ-carotene and what biosynthetic relationship exists between this poly-*cis* carotene and its possible precursors [3,8].

Table 1. λ_{max} of γ-carotene, poly-*cis*-γ-carotene and some natural and synthetic isomers [4–7]

Isomer	Source	λ_{max} I_2 Catalysis Before	After
all-*trans*-γ-Carotene	*Mimulus longiflora*	436, 461–2, 493	434, 458, 486–7
pro-γ-Carotene	*Pyracantha angustifolia*	434, 457	434, 457, 487–8
cis-15-15′-γ-Carotene	Synthesis	432, 457, 486	
γ-Carotene	High-beta tomato*	431, 456, 486	428, 453, 482
γ-Carotene	Carrots	431, 456, 486	428, 454, 482
γ-Carotene	Tangerine tomato*	431, 456, 486	428, 454, 482
poly-*cis*-γ-Carotene	Tangerine tomato*	428, 453	428, 453, 482

* *Lycopersicon esculentum*

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*Contribution No. 1551 of Rhode Island Agricultural Experiment Station.