Phytochemistry, 1971, Vol. 10, pp. 1595 to 1601. Pergamon Press. Printed in England.

A NEW $C_{40}H_{56}$ CAROTENE WITH A TERMINAL METHYLENE GROUP*

N. ARPIN, J.-L. FIASSON and M. P. BOUCHEZ-DANGYE-CAYE
Département de Biologie V&g&tale,
Laboratoire de Mycologie associe au C.N.R.S.
Service de Phytochimie et Phytophysiologie, Université de Lyon, France
and
G. W. FRANCIS and S. LIAAEN-JENSEN
Organic Chemistry Laboratories, Norwegian Institute of Technology,
University of Trondheim, Norway

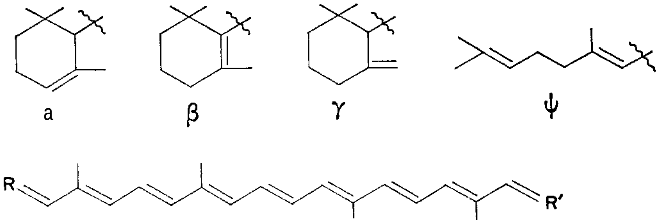
(Received 27 September 1970)

Abstract-The structure of a new $C_{40}H_{56}$ carotene isolated from the discomycete *Caloscypha fulgens* (Pers.) Boud. is shown by spectral methods to be β,γ-carotene (VII). This is the first demonstration of a naturally occurring carotenoid with a terminal methylene group.

INTRODUCTION

A TERMINAL methylene group is a rather common structural feature among the terpenes, but it has so far not been demonstrated in naturally occurring carotenoids, of which there are some three hundred compounds.’ We now report on the first carotenoid with exocyclic methylene group obtained from a natural source.

The $C_{40}H_{56}$ carotenes have hitherto comprised α,α-carotene (I, E-carotene), α,β-carotene (II, u-carotene), @-carotene (III, p-carotene), a, #-carotene (IV, δ-carotene),β, #-carotene (V, y-carotene) and ψ,ψ-carotene (VI, lycopene),[2] Scheme 1. The new nomenclature rules[3] are here used with the former designations in parenthesis.



I R = R ' = a
II R=α, R′=β
III R=R′=β
IV R=α, R′=ψ
V R=β, R′=ψ
VI R=R′=ψ
VII R=β, R′=γ

SCHEME 1.

The new compound &y-carotene (VII) is the seventh member of the $C_{40}H_{56}$ carotenes.

---

* Part VI in the series "Fungal Carotenoids"; for Part V see *C.R. Acad. Sci. Paris 265* Série D, 1083 (1967). Also Part XVIII in the series "Recherches chimiotaxinomiques sur les champignons" ; for Part XVII see *"Evolution of Hiphen Basidiomycetes"*, Knoxville, U.S.A. (in press).

[1] 0. STRAUB, in 0. ISLER, *Carotenoids, A Progress Report,* Birkhluser, Basel (1971,.

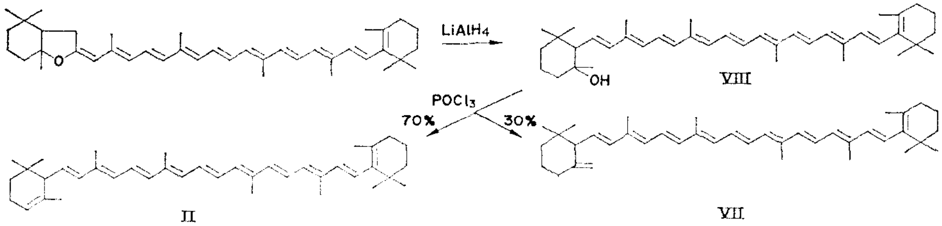
[2] U. SCHWIETER, H. R. BOLLIGER, L. CHOPARD-DIT-JEAN, G. ENGLERT, M. KOFLER, A. KÖNIG, C. v.PLANTA, R. RÜEGG, W. VETTER and 0. ISLER, *Chimia* 19, 294 (1965).

[3] IUPAGIUB Commission on Biochemical Nomenclature, IUPAC Commission on the Nomenclature of Organic Chemistry. Tentative Rules for the Nomenclature of Carotenoids (1970).

RESULTS AND DISCUSSION

The carotenoid composition of the discomycete ***Caloscypha fulgens*** (Pers.) Boud. has been analysed by Arpin[4] and found to contain a mixture of carotenes. An unidentified component, provisionally designated P444 and comprising *ca.* *8* per cent of the total carotenoid, exhibited the same electronic spectrum as &carotene (II), but was slightly more strongly adsorbed.

Structure VII has been assumed for a minor product, with spectroscopic (visible light) and chromatographic properties resembling those of P444, obtained on dehydration of the tertiary alcohol VIII with phosphorus oxychloride,[5] see Scheme 2.



SCHEME 2.

We now report the isolation of P444 in the pure state from C. *fulgens* and present evidence for structure VII (β,γ-carotene) for this new carotene.

β,γ-Carotene (VII) crystallized as needles from benzene-methanol, yield 39 mg. VII melted (176-177" uncorr.) in the same region as the structurally closely related &-carotene (II, m.p.h 187-188" corr.)[6] and β,β-carotene (III, m.p. 183" corr.).[7]

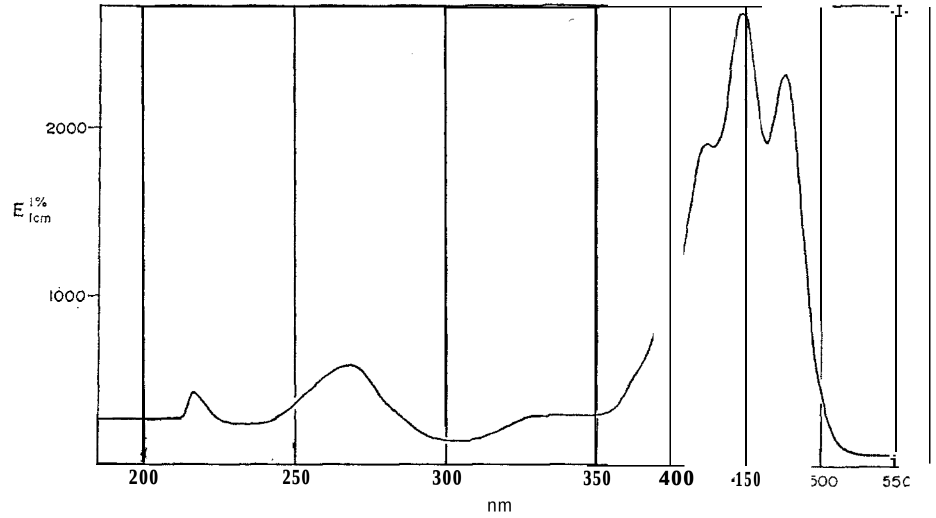


FIG. 1. ELECTRONIC S &-CAROTENE (VII) IN PETROLEUM b.p. 40–60°.

---

[4] RPIN *Discomycètes: Essai Chimiotaxinomique p.* 102, Université de Lyon, (1968).

[5] S. HERTZBERG and S. LIAAEN JENSEN, *Phytochem.* 6, 1119 (1967).

[6] KARRER and ,0. W *Acta* 16, 642 (1933).

[7] R. KUHN BROCKMANN, *Ber.* 66,408

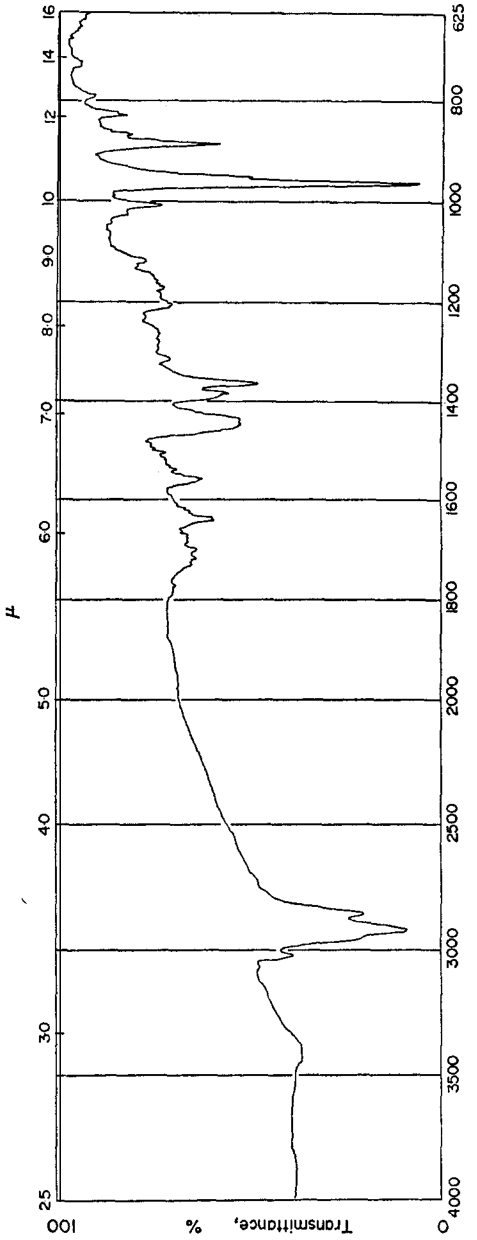


FIG. 2. I.r. SPECTRUM (KBr) OF β,γ-CAROTENE (VII).

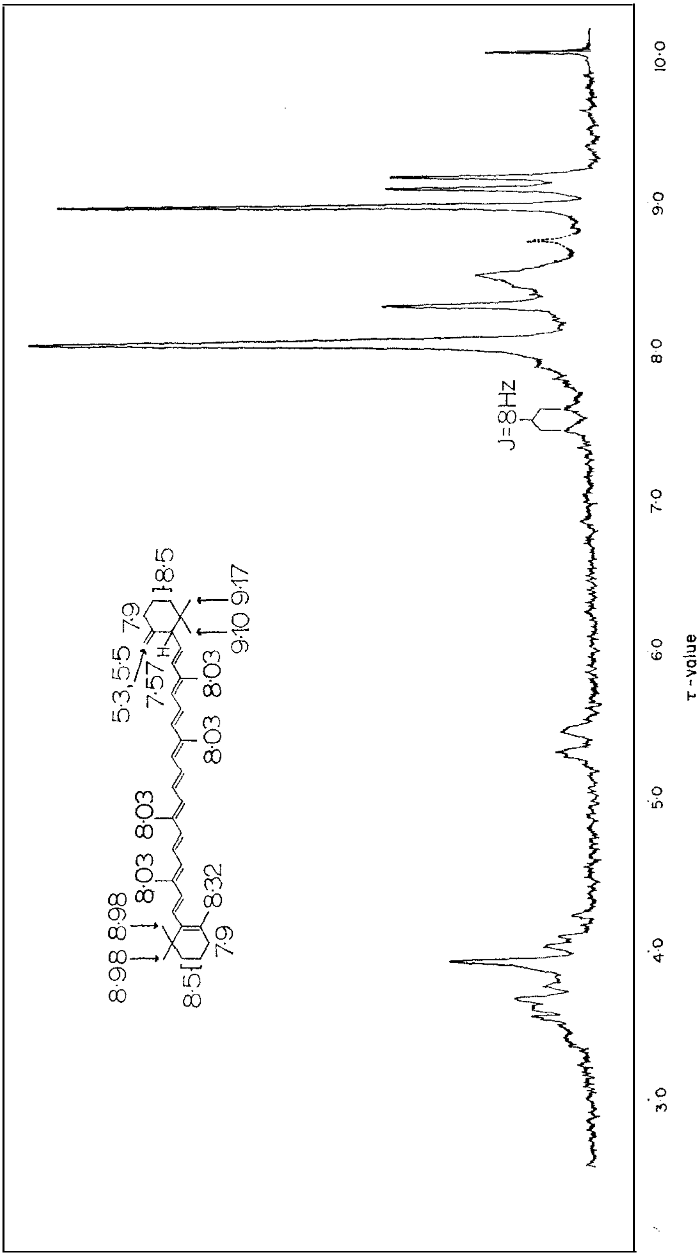


FIG. 3. NMR SPECTRUM ($CDCl_3$) OF β,γ-CAROTENE (VII).

The electronic spectrum recorded in petroleum (Fig. 1) corresponded closely to that of a,#?-carotene (II), demonstrating the same chromophore in VII and II.

The i.r. spectrum of VII (Fig. 2) resembled those of the common carotenes except for strong, characteristic absorption at 889 cm^{-1}, ascribed to $R_1R_2C{=}CH_2$.[8]

The NMR spectrum of VII including signal assignments is presented in Fig. 3. The presence of a β-type end group was inferred from signals at τ 8·98 (singlet, 6H, gem. dimethyl) and τ 8·30 (singlet, 3H, end-of-chain methyl in β-ring).[9] Also the second end group was obviously cyclic as judged by methyl signals at τ 9·17 (singlet, 3H) and τ 9·10 (singlet, 3H). Together with broad singlets at τ 5·31 (1H) and 5·46 (1H) ascribed to a $R_1R_2C{=}CH_2$ grouping with magnetically non-equivalent protons and small coupling constant[10] (W_H = *ca.* 4 Hz) and a doublet at τ 7·57 (1H, J = 8 Hz) attributed to the methine proton at C-6', this strongly suggests the presence of a y-type (new nomenclature)[3] end group. Furthermore, the other features of the NMR spectrum are compatible with structure VII. Thus the four in-chain methyl groups give rise to a singlet at τ 8·03 (12H), non-allylic methylene groups (8H) and allylic methylene groups (4H) give rise to multiplets centered at τ 8·5 and *ca.* 7·9 respectively, and the olefinic protons of the polyene chain (14H) cause signals in the τ 3·4–4·2 region. The singlet at τ 3·86 for β,β-carotene (III) caused by the magnetically equivalent 7,8,7',8'-protons,[9] here observed at τ 3.89, corresponds to only *ca.* two protons as required by structure VII. The presence of only nine methyl groups is in further agreement with structure VII.

High resolution mass spectrometry showed that the molecular ion of VII corresponded to the formula $C_{40}H_{56}$. The M-92, M-106 and M-158 ions observed for other carotenes[10]

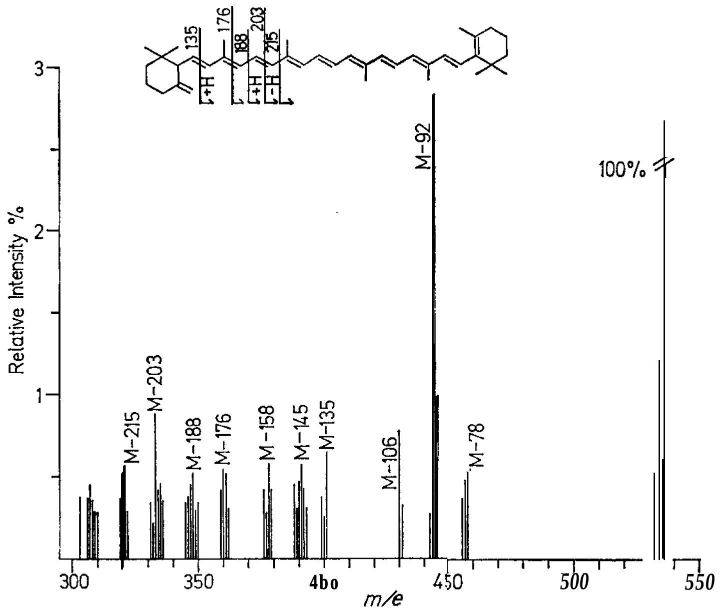


Fig. 4. Mass spectrumof β,γ-carotene (VII).

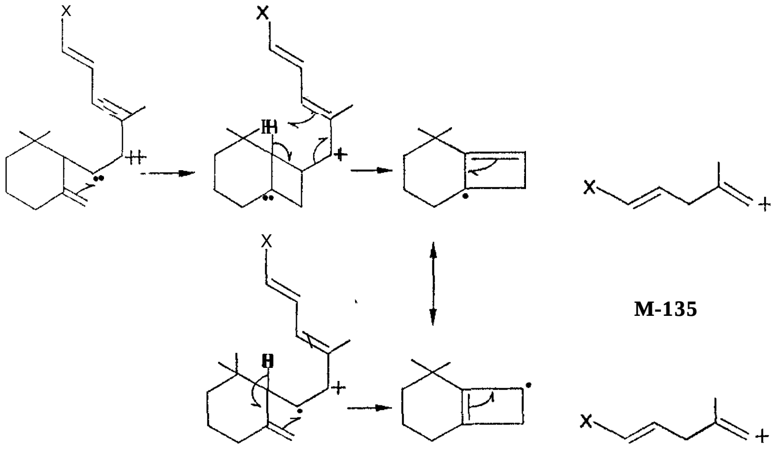
---

[8] L. J. Bellamy, *The Infra-red Spectra of Complex Molecules*, p. 34, 2nd edition, Methuen, London (1964).

[9] U. Schwieter, G. Englert, N. Rigassi and W. Vetter, *J. Pure Appl. Chem. 20, 365 (1969).*

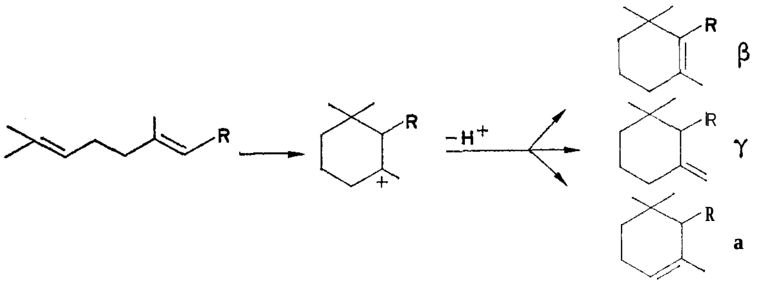
[10] L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 277, Pergamon Press, Oxford (1969).

were recorded, and the M-92/M-106 intensity ratio (3·66) was within the range found for bicyclic carotenoids with nine conjugated double bonds in the central acyclic chain.[11] A number of ions ascribed to in-chain cleavage were recorded (see Fig. 4). Some of these cleavages involved hydrogen transfer. Whereas cleavage of the C(7)-C(8) double bond in carotenoids containing unsubstituted β-rings results in an abundant M-137 ion, requiring hydrogen transfer to the smaller fragment,[12] an M-135 peak is observed for VII and is most likely associated with the y-ring. Taking into account that u-rings appear to cause hydrogen transfer in the same direction as β-rings,[12] a possible concerted mechanism is suggested in Scheme 3 for the formation of the M-l 35 ion from VII. Alternatively stabilization of the exocyclic double bond could be regarded as a separate step, followed by cleavage through a common six-membered transition state.



Scheme 3.

The new carotene (VII) is expected to be optically active. Its optical properties and stereochemical relationship to a&carotene (II, now known to possess 6S-configuration)[13] will be dealt with elsewhere.[14]



Scheme 4.

---

[11] C. R. Enzell, G. W. Francis and S. Liaaen-Jensen, *Acta Chem. Scand.* 22, 1054 (1968).

[12] C. R. Enzell, G. W. Francis and S. Liaaen-Jensen, *Acta Chem. Scand.* 23, 727 (1969).

[13] C. H. Eugster, R. Buchecker, C. Tscharner, G. Uhde and G. Ohloff, *Helv. Chim. Acta* 52, 1729 (1969).

[14] G. Borch and S. Liaaen-Jensen, to be published.

Concerning the biosynthesis of VII, it occurs together with other aliphatic, monocyclic and bicyclic carotenes, none of which appears to have an α-ring.[4] It therefore appears that the cyclizing enzymes operating in the carotenogenesis of this discomycete either lead to P-rings or to y-rings with exocyclic double bond, both of which may have a common carbonium ion intermediate, Scheme 4.

The similarity in visible light absorption properties and chromatographic behaviour of carotenoids with *α*- and y-type (new nomenclature)[3] end groups may be the reason why this structural element has previously escaped detection.

EXPERIMENTAL

General methods and the spectral equipment used were those commonly employed in our laboratories.[4,15]

Biological material. Caloscypha fulgens (Pers.) Boud., wet weight 3·0 kg, was collected in the Claveisolles forest (Rhône) in May 1970 and stored deep-frozen.

Isolation of VIZ. The fungi were carefully cleaned of all foreign matter and washed with water. The fungi were repeatedly extracted with acetone at room temp. until the final extract was colourless; total yield 325 mg (based on $E^{1\%}_{cm}$ = 2500 at A,,, in acetone) carotenoids corresponding to 0·18 per cent of the acetone-extracted residue. The acetone extract was concentrated and the carotenoids transferred to petroleum on admixture with brine. The petroleum extract was washed, concentrated and ergosterol precipitated at 4” and removed by filtration. The process was repeated twice. The supernatant was submitted to standard saponification in ether-methanol containing 5% KOH. The unsaponifiable matter was chromatographed on columns of neutral alumina, activity grade 2.[16] β,β-Carotene (III) required 2% and VII 3 % ether in petroleum for elution. VII, comprising 13 per cent of the total carotenoid, was crystallized from benzene-methanol; yield 39 mg.

Characterization of β,γ-carotene (VII). VII had m.p. 176-177” uncorr. using abbreviated thermometer. In co-chromatography tests on aluminium oxide paper[17] (5 % ether in petroleum) a&carotene (II, isolated from carrots) and VII had R_fs 0·49 and 0·38 respectively. VII was completely epiphasic when partitioned between petroleum and 95% aq. methanol, and gave no new products when treated with 10% KOH in ether-methanol overnight or with $LiAlH_4$ in dry ether.

Abs. max. in petroleum b.p. 40–60° (Fig. 1) were at (400), 420, 448 ($E^{1\%}_{1cm}$ = 2740) and 478 nm; characteristic ν_{max} (KBr) 1640, 1560 (double bonds), 1450 (CH_2), 1385 and 1365 (methyl, gem. dimethyl), 1010, 962 (*trans.* disubst. double bonds), 888 ($R_1R_2C{=}CH_2$) and 830 (trisubst. double bonds) cm-‘, see Fig. 2. For T-values ($CDCl_3$) see Fig. 3, and mass spectrum Fig. 4: *m/e* 536.4385 (M, calc. for $C_{40}H_{56}$ 536·4372), M-78, M-92, M-106, M-135, M-158, 145, 69.

***Acknowledgements*-G.W.F.** was supported by a grant from the Norwegian Institute of Technology.

---

[15] A. J. Aasen and S. Liaaen Jensen, *Acta Chem. Scand.* **20,** 1970 (1966).
[16] H. Brockmann and H. Schodder, *Ber.* **74,** 73 (1941).
[17] A. Jensen, *Acta Chem. Scand.* **13,** 1259 (1959).