782. *Carotenoids and Related Compounds. Part IX.* The Structures of Capsanthin and Capsorubin.*

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The structures of capsanthin (I; R = d) and capsorubin (II; R = d) have been established. These pigments constitute a new class of carotenoid containing five-membered rings.

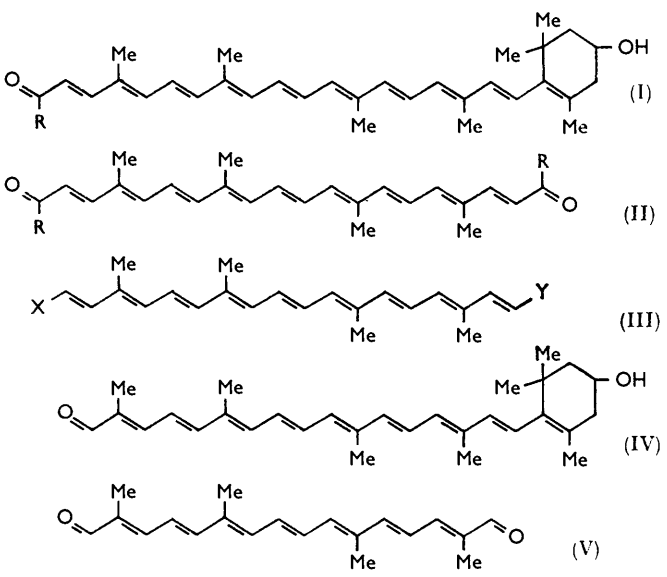
Capsanthin and, in much smaller amounts, capsorubin occur together in red peppers (*Capsicum annuum*),[1] and there is now strong evidence that they are both produced in Nature from zeaxanthin (III; X = Y = a) through the epoxides antheraxanthin (III; X = b, Y = a) and violaxanthin (III; X = Y = b), respectively.[2] Previous work on the structures of capsanthin and capsorubin has shown that the former contains a decaenone (I),[1,3] and the latter a nonaenedione (II),[4,5] chromophore. Alkaline degradation of capsanthin to β-citraurin (IV),[6] and of capsorubin to crocetindial (V),[5] establishes the nature of the polyene systems, and shows that capsanthin retains one end of the zeaxanthin molecule intact.

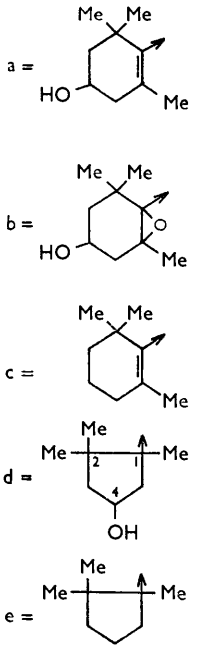
The scarcity of capsorubin has greatly hampered the investigation of this compound, and much reliance has been placed on analogy with capsanthin. It has often been assumed that the two end groups of capsorubin are identical with each other, and with one end of capsanthin. Evidence is presented below that substantiates these assumptions. The structural problem with both carotenoids therefore relates to the $C_{(3)}$ hydroxylated substituent (R in I and II) attached to the carbonyl groups. Various proposals have been made, the most strongly advocated being (VI), (VII),[7] (VIII),[8] and (IX) [9]. Of these the first two have been disproved synthetically.[5] The others are difficult to reconcile

* Part VIII, *J.*, 1959, 4058.

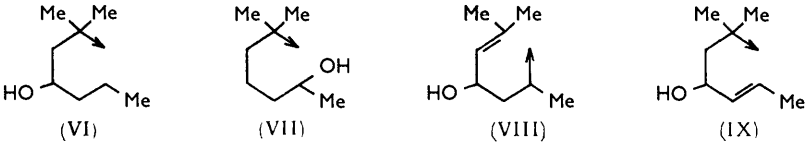
[1] Zechmeister and Cholnoky, *Annalen*, 1934, **509**, 269; 1935, **516**, 30.
[2] Cholnoky, Györgyfy, Nagy, and Pánczél, *Acta Chim. Acad. Sci. Hung.*, 1955, **6**, 143.
[3] Karrer and Jucker, *Helv. Chim. Acta*, 1944, **27**, 1588.
[4] Ahmad and Weedon, *J.*, 1953, 3286, 3815.
[5] Warren and Weedon, *J.*, 1958, 3972.
[6] Zechmeister and Cholnoky, *Annalen*, 1937, **530**, 291.
[7] Entschel, Eugster, and Karrer, *Helv. Chim. Acta*, 1956, **39**, 1263.
[8] Cholnoky, Szabó, and Szabolcs, *Annalen*, 1957, **606**, 194.
[9] Cholnoky and Szabolcs, *Naturwiss.*, 1957, **19**, 513.

with the evidence that neither capsanthin nor capsorubin contains an (acyclic) γ-hydroxy-ketone grouping.[5] The structure (VIII) is also inconsistent with the view that both pigments are tertiary, rather than secondary, alkyl ketones.[5]





To define the nature of the hydroxyl group in the " unknown " end groups, the oxidation of capsanthin was studied. Under suitable conditions, Oppenauer oxidation with aluminium t-butoxide and acetone gave a hydroxy-diketone, capsanthone, in 20% yield.



Its spectral properties were very similar to those of capsanthin, except for an additional strong band at 1739 cm.$^{-1}$ attributable to a cyclopentanone.[10] The presence of one hydroxyl group, indicated by a band at 3630 cm.$^{-1}$ (ε 40) with half the intensity of the corresponding band in zeaxanthin (3625 cm.$^{-1}$, ε 90), was confirmed by preparation of a monoacetate. Capsanthone was recovered after treatment with alcoholic sodium ethoxide, and a β-diketone structure is therefore improbable.

That oxidation of capsanthin involved the " unknown " end group was borne out by subjecting zeaxanthin and capsorubin to the same treatment. The former yielded no C_{40} ketone, but the latter gave (5%) capsorubone, with carbonyl absorption bands in the same positions as those of capsanthone.

Reduction of capsanthin to the corresponding decaenetriol,[5,11,12] and re-oxidation of the latter to capsanthin with chloranil,[5] have been reported previously. Reduction of capsanthone with lithium aluminium hydride gave a mixture of decaenetriols. Selective oxidation of the allylic hydroxyl groups with chloranil and chromatography then yielded

[10] Bellamy, " The Infra-Red Spectra of Complex Molecules," Methuen, London, 1958.
[11] Karrer and Hübner, *Helv. Chim. Acta*, 1936, **19**, 476.
[12] Goodwin, Land, and Sissins, *Biochem. J.*, 1956, **64**, 486.

two decaenones which were doubtless epimers differing in the configuration of the hydroxy-groups formed on reduction of the keto-group in the cyclopentane ring. One of the products was identical with capsanthin, the other exhibited a carbonyl absorption band at shorter (18 cm.$^{-1}$) frequency and of lower intensity. It may be concluded that capsanthin also contains a cyclopentane ring, and tentatively that the two oxygenated substituents of this ring are *trans* to one another.

Further information concerning the structures of the two carotenoid ketones was obtained from a study of their nuclear magnetic resonance spectra in the 9·5—6·5 p.p.m. region. That of capsorubin proved suprisingly simple and will therefore be considered first. It showed four well-defined bands, attributable to methyl groups, at 9·02, 8·77, 8·65, and 8·02 p.p.m. in the approximate ratios 1 : 1 : 1 : 2. The band at 8·02 is typical of " in-chain " methyl groups of the type CH:CH·CMe:CH in polyene systems.[13] Since there are four such groups in capsorubin the other bands must each represent two equivalent methyl groups. This is readily understood if the two end groups of capsorubin are the same, each containing three methyl groups. The bands are sharp, and since their separations (cycles/sec.) are proportional to the strength of the applied magnetic field, all the methyl groups must be attached to fully substituted aliphatic carbon atoms.

The nuclear magnetic resonance spectrum of capsanthin showed methyl bands at 9·02, 8·95, 8·77, 8·65, 8·3, and 8·02 p.p.m. in the approximate ratios 1 : 2 : 1 : 1 : 1 : 4. The bands at 8·95 and 8·3 are also observed in zeaxanthin [13] and are due to the methyl groups on the cyclohexene ring common to both carotenoids; the band at 8·02 may again be ascribed to the four " in-chain " methyl groups. The three remaining bands are identical in position with, but only half as intense as, those observed for the non-allylic methyl groups in capsorubin. This provides very strong support for the view that one end group of capsanthin is identical with those of capsorubin. The simultaneous occurrence of three methyl bands at 9·02, 8·77, and 8·65 has not been encountered in a broad survey of the nuclear magnetic resonance spectra of other natural and synthetic carotenoids.[13] The spectrum of capsanthone was very similar to that of capsanthin, indicating that the same (cyclopentane) basic structure is present in both.

The above chemical and spectral evidence indicates that the characteristic ends of capsanthin and capsorubin are cyclopentyl groups possessing three methyl substituents, each of which is on a fully substituted carbon atom, and also a secondary hydroxyl substituent. One methyl group must therefore be sited at $C_{(1)}$*, and the other two must constitute a *gem*-dimethyl group. The hydroxyl group is most probably at $C_{(3)}$ or $C_{(4)}$, since capsanthone shows none of the properties of a β-diketone. Cyclopentane structures have not previously been encountered in naturally occurring carotenoids, but their presence in the paprika ketones can be readily explained by postulating a pinacolic rearrangement of the epoxides (or related derivatives) of zeaxanthin from which they are believed to be formed in Nature.[2] It can therefore be concluded that capsanthin has structure (I; R = d) and capsorubin (II; R = d); these formulations are in complete accord with all the evidence given above, and with the extensive analytical results and revised molecular formulæ of Cholnoky *et al.*[8] Although the behaviour towards chloranil of the hydroxy-compounds (capsanthol and capsorubol) formed by reduction of these carotenoids with potassium borohydride has previously been contrasted with that of the products from authentic (acyclic) γ-hydroxy-ketones,[5] this difference is not surprising in view of the ring structures now detected in the natural compounds. The reported dehydration of capsanthol, and the resistance of capsorubol, on treatment with hydrochloric acid [9] is also readily understood.

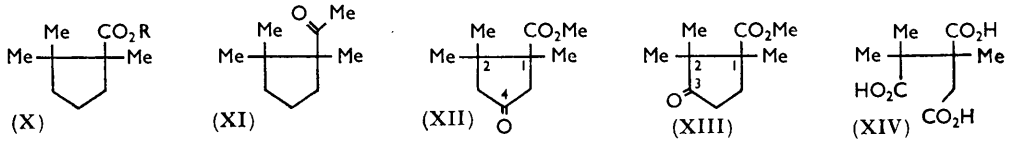
Mention must also be made of a few reports which appear to be inconsistent with structures (I and II; R = d). In support of structure (VII) for one end group of

* Geneva numbering; $C_{(1)}$ is equivalent to $C_{(5)}$ on the standard carotenoid numbering.

[13] Barber, Davis, Jackman, and Weedon, *J.*, 1960, 2870.

capsanthin, Karrer *et al.*[7] cited the formation of propionic, butyric, and valeric acid on oxidation of dehydroxyperhydrocapsanthin with chromic acid; however, no yields were given, and the acids were identified only by paper chromatography. Their main product was apparently a C_9 acid which is in good agreement with structure (I; R = d) for capsanthin. Cholnoky *et al.*[8] have concluded from micro-hydrogenation studies that capsanthin and its esters contain eleven double bonds and not ten as previously reported; however, in our experience, catalytic hydrogenation of capsanthin is accompanied by at least partial reduction of the keto-group. The same authors obtained 0·3—0·4 mol. of acetone on oxidation of capsanthin, again contrary to earlier reports, and therefore favoured the isopropylidene structure (VIII) for the end group; however, the yield of acetone is considerably lower than that obtained from authentic isopropylidene compounds, and it is known that other structures can give rise to acetone under the experimental conditions used. The doubtful significance of these results is apparently recognised by the subsequent proposal of (IX) for the end group.[9] That neither capsanthin nor capsorubin contains an isopropylidene end group is unequivocally shown by the nuclear magnetic resonance spectra.

Biogenetic schemes, based on carotenoids other than zeaxanthin, are conceivable which would allocate the hydroxyl group to position 3 or 5 in the five-membered ring rather than to position 4. However, confirmation of the structures assigned to the characteristic end group of capsanthin and capsorubin was obtained in the following way. Treatment of 1,2,2-trimethylcyclopentanecarboxylic (camphonanic) acid[14] (X; R = H) with methyl-lithium gave the methyl ketone (XI), which on condensation with apo-2-carotenal and crocetindial (V) gave dehydroxy-capsanthin and -capsorubin (*e.g.*, II; R = e) respectively. The nuclear magnetic resonance spectra of these products included three bands (9·15, 8·89, 8·81 p.p.m.) due to the methyl groups on the cyclopentane rings. By comparison the three bands (9·02, 8·77, 8·65 p.p.m.) attributable to the methyl groups on the cyclopentanone ring of capsanthone occurred at significantly lower fields. This deshielding due to the extra keto-group is identical (within experimental error) with that found on comparing the spectra of the methyl ester (X; R = Me) (9·14, 8·93, 8·87) and its 4-oxo-derivative[15] (XII) (8·98, 8·83, 8·70), but different from that due to the 3-keto-group in the ester[14] (XIII; R = Me) (9·06, 8·96, 8·77 p.p.m.). The keto-group in the five-membered ring of capsanthone, like that in (XII), must therefore be symmetrically placed with respect to the three methyl groups.



In an interesting paper submitted independently of our preliminary publication,[16] Entschel and Karrer[17] have arrived at some of the same conclusions. They describe the Oppenauer oxidation of capsanthin and capsorubin, and the degradation of di-*O*-acetylcapsorubin to an acid, $C_9H_{16}O_3$, which they believe to be a hydroxycamphonanic acid. They conclude that capsanthin and capsorubin have structures (I and II; R = d), or are isomers of these with hydroxyl groups at other positions of the five-membered rings. The identity of the three end groups (R in I and II) was not clearly established, and the nature and position of the alkyl substituents was deduced from biogenetic arguments only.

More recently Cholnoky and Szabolcs[18] reported briefly the oxidative degradation of capsorubin to a mixture of acids which included αα-dimethylsuccinic, trimethylsuccinic,

[14] Appel, *Z. phys. Chem.*, 1933, **218**, 202.
[15] Cooper and Weedon, unpublished results.
[16] Barber, Jackman, Warren, and Weedon, *Proc. Chem. Soc.*, 1960, 19.
[17] Entschel and Karrer, *Helv. Chim. Acta*, 1960, **43**, 89.
[18] Cholnoky and Szabolcs, *Experientia*, 1960, **16**, 483.

αα'β-trimethylglutaric, and the tricarboxylic acid (XIV) (camphoronic acid). In conjunction with our evidence for the identity of the two end groups, these results provide strong confirmation of our main structural conclusions.

Experimental

As far as possible, operations were carried out in an inert atmosphere, usually of nitrogen. Alumina for chromatography was pre-treated as described by Cheeseman *et al.*[19] and was grade IV on the Brockmann and Schodder [20] activity scale.

M. p.s were determined in evacuated capillary tubes unless otherwise stated; those determined on a Kofler block are corrected.

Visible and infrared light absorption data were determined for benzene and chloroform solutions, respectively.

Nuclear magnetic resonance spectra were determined for chloroform or deuterochloroform solutions at 40 and 56·4 Mc./sec., as previously described.[13]

Capsanthone.—A mixture of capsanthin (200 mg.; pure) and aluminium t-butoxide (8 g.) in acetone (200 c.c.) and benzene (200 c.c.) was heated under reflux for 36 hr., cooled, and shaken with N-sulphuric acid (2 × 200 c.c.). The organic layer was washed with water and aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure, finally at $50°/10^{-2}$ mm. Chromatography of the residue in benzene on alumina, collection of the main band, evaporation, and crystallisation from aqueous methanol gave *capsanthone* (40 mg.) as red rhombs, m. p. 167° (Found: C, 82·6; H, 9·45; O, 8·6. $C_{40}H_{54}O_3$ requires C, 82·45; H, 9·35; O, 8·25%), $\lambda_{max.}$ 489 mμ (ε 105,000), inflection 516 mμ, $\nu_{max.}$ 3630 (ε 40), 1739 (ε 480), 1664 (ε 210), 1576, 1546, 1505, 1004, 985, and 970 $cm.^{-1}$. Reduction of the reaction time or of the proportion of butoxide lowered the yield appreciably. Attempts to use slightly impure capsanthin were also unsatisfactory. In some experiments small amounts of a *cis*-capsanthone ($\lambda_{max.}$ 478, 367 mμ) were observed which yielded the above " all-*trans* "-isomer on irradiation in benzene in the presence of a trace of iodine.

A solution of capsanthone (25 mg.) and acetic anhydride (1 c.c.) in pyridine (5 c.c.) was warmed gently, kept for 1 hr., and then poured into water. Isolation of the product in the usual way, chromatography from benzene on alumina, and crystallisation from aqueous methanol gave the *acetate* (14 mg.) as red rhombs, m. p. 152° (Found: C, 80·5; H, 9·3; O, 9·9. $C_{42}H_{56}O_4$ requires C, 80·8; H, 9·0; O, 10·2%), $\lambda_{max.}$ 489 mμ (ε 99,000), inflection 515 mμ, $\nu_{max.}$ 1736, 1725 (sh), 1661 (ε 190), 1578, 1549, 1507, 1006, 980, 971 $cm.^{-1}$. Acetylation of capsanthone with acetyl chloride in pyridine gave lower yields of the acetate.

Capsanthone (6 mg.) was recovered (comparison by infrared and visible spectra, and mixed chromatogram) after treatment with sodium methoxide (from 15 mg. of sodium) in methanol (2 c.c.) at 20° for 48 hr.

Capsorubone.—A mixture of capsorubin (51 mg.) and aluminium t-butoxide (1 g.) in acetone (50 c.c.) and benzene (50 c.c.) was heated under reflux for 36 hr. Isolation of the crude product, chromatography from benzene on alumina, collection of the main red band, and evaporation gave capsorubone (*ca.* 5 mg.), $\lambda_{max.}$ 524, 491 mμ, $\nu_{max.}$ 1735, 1661 $cm.^{-1}$, $\varepsilon_{1739}/\varepsilon_{1664} = 2·2$ (cf. 2·3 for capsanthone).

Conversion of Capsanthone into Capsanthin.—(i) A solution of capsanthone (31 mg.) in tetrahydrofuran (10 c.c.) was added to one of lithium aluminium hydride (40 mg.) in the same solvent (10 c.c.). The mixture was stirred at 20° for 30 min. Methanol was added to decompose the excess of hydride. The solution was diluted with benzene (20 c.c.) and then washed with 0·5N-sulphuric acid and aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure, giving a crude mixture of decaenetriols, $\lambda_{max.}$ 484 and 458 mμ (Warren and Weedon [5] give $\lambda_{max.}$ 483 and 455 mμ for capsanthol).

A solution of the crude triols and chloranil (30 mg.) in benzene (7 c.c.) was kept at 20° for 24 hr., then poured on a column of alumina. Development of the chromatogram with 0·2% of ethanol in benzene gave three main bands which were collected. The least strongly adsorbed band, with $\lambda_{max.}$ 490, 463 mμ, was not examined further. The second band was evaporated, giving a solid (*ca.* 4 mg.), $\lambda_{max.}$ 484, inflection 516 mμ, no " *cis*-peak," $\nu_{max.}$ 3600, 3450, 1646, and 1033 $cm.^{-1}$, $\varepsilon_{1033}/\varepsilon_{1646}$ *ca.* 5·5. It separated from both capsanthin and capsanthone in mixed chromatograms. Evaporation of the third (most strongly adsorbed) band gave a solid (4 mg.),

[19] Cheeseman, Heilbron, Jones, and Weedon, *J.*, 1949, 3120.
[20] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

m. p. 170°, $\lambda_{max.}$ 484, inflection 515 mμ. Its infrared absorption spectrum ($\nu_{max.}$ 3600, 1664 cm.$^{-1}$; $\varepsilon_{1033}/\varepsilon_{1664}$ = *ca.* 4·1)was identical with that of natural capsanthin, and no separation was observed in a mixed chromatogram.

(ii) A solution of potassium borohydride (11 mg.) and capsanthone (9 mg.) in methanol (2 c.c.) was kept at 20° for 14 hr. Reduction being incomplete, further borohydride (10 mg.) was added. After 3 hr. a specimen of the solution diluted with benzene had $\lambda_{max.}$ 486 and 458 mμ. The methanol solution was treated with 2N-sodium hydroxide (0·2 c.c.) and kept for 2 hr. Benzene was added and the solution was washed with water, dried ($MgSO_4$), and evaporated.

Oxidation of the crude product in benzene (7 c.c.) with chloranil (11 mg.) and isolation as described in the previous experiment gave: (i) a solid, $\lambda_{max.}$ 489 mμ, m. p. 162—164° (Kofler), undepressed on admixture with capsanthone, from which it did not separate in a mixed chromatogram; (ii) a solid, m. p. 181—185° (Kofler), $\lambda_{max.}$ 484, inflection 515 mμ, which separated from both capsanthin and capsanthone in mixed chromatograms; and (iii) a solid, $\lambda_{max.}$ 483, inflection 516 mμ, which did not separate from capsanthin in a mixed chromatogram.

1-*Acetyl*-1,2,2-*trimethylcyclopentane* (XI).—A solution of 1,2,2-trimethylcyclopentanecarboxylic (camphonanic) acid [14] (8·0 g.) in ether (20 ml.) was added during 30 min. to one of methyllithium (from 3·0 g. of lithium) in ether (100 ml.). The resulting solution was stirred at 20° for 4 hr. and then under reflux for 12 hr. Isolation of the neutral product in the usual way and distillation gave a fraction (4·6 g.), b. p. 90—92°/19 mm., m. p. 40—45°. Since gas–liquid chromatography revealed the presence of *ca.* 10% of an impurity with a longer retention time, the crude product was purified by chromatography on alumina and gave the *ketone*, b. p. 78°/12 mm., m. p. 50° (Found: C, 77·7; H, 11·75; O, 10·25. $C_{10}H_{18}O$ requires C, 77·9; H, 11·75; O, 10·35%), $\nu_{max.}$ 1695 cm.$^{-1}$, τ 7·92 ($CO{\cdot}CH_3$), 8·44, 8·89, 8·93, and 9·14 p.p.m. The 2,4-*dinitrophenylhydrazone* crystallised from chloroform–methanol in plates, m. p. 141° (Found: C, 57·4; H, 6·7; N, 16·9. $C_{16}H_{22}O_4N_4$ requires C, 57·45; H, 6·65; N, 16·75%). The semicarbazone was formed in low yield and crystallised from aqueous alcohol in needles, m. p. 205—206°.

4,8,13,17-*Tetramethyl*-1,20-*di*-(1,2,2-*trimethylcyclopentyl*)*eicosa*-2,4,6,8,10,12,14,16,18-*nonaene*-1,20-*dione*(II; R = e).—A mixture of the preceding ketone (1·0 g.), crocetindial [21] (100 mg.), and 5% ethanolic potassium hydroxide (6·0 c.c.) was warmed and shaken occasionally. After 2 days the mixture was refluxed for 30 min., diluted with ethanol (6 c.c.), and kept at −10° overnight. The purple crystals (125 mg., 65%) which had separated were collected and had m. p. 200—205°. Recrystallisation from benzene–methanol gave the *polyene diketone*, m. p. 205° (Found: C, 84·5; H, 9·9; O, 5·9. $C_{40}H_{56}O_2$ requires C, 84·5; H, 9·9; O, 5·6%), $\lambda_{max.}$ 458, 486, and 520 mμ (10^{-3} ε 88, 126, and 115 respectively), $\nu_{max.}$ 1661 cm.$^{-1}$ (ε 440), τ 8·03, 8·81, 8·89, and 9·15 p.p.m.

4,8,13,17-*Tetramethyl*-19-(2,6,6-*trimethylcyclohex*-1-*enyl*)-1-(1,2,2-*trimethylcyclopentyl*)*nonadeca*-2,4,6,8,10,12,14,16,18-*nonaen*-1-*one*.—A mixture of apo-2-β-carotenal [22] (200 mg.), 1-acetyl-1,2,2-trimethylcyclopentane (1·0 g.) and 5% ethanolic potassium hydroxide (15 c.c.) was warmed and shaken occasionally. After 2 days the mixture was boiled under reflux for 30 min., cooled, and diluted with light petroleum (b. p. 60—80°) (250 c.c.). The petroleum solution was washed with aqueous methanol (1 : 9) and then with water, dried, and evaporated. Chromatography of the residue in benzene on alumina, isolation of the main red band, evaporation, and crystallisation of the residue from benzene–methanol gave the *polyene ketone* (25 mg.) as needles, m. p. 164—165° (Found: C, 86·8; H, 10·5. $C_{40}H_{56}O$ requires C, 86·9; H, 10·2%), $\lambda_{max.}$ 483 mμ (ε 101,000), $\nu_{max.}$ 1667 cm.$^{-1}$ (ε 200), τ 8·03, 8·28, 8·82, 8·89, 8·97, 9·15 p.p.m.

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South Kensington, London, S.W.7. [*Received, May 8th*, 1961.]

[21] Isler, Gutmann, Lindlar, Montavon, Rüegg, Ryser, and Zeller, *Helv. Chim. Acta*, 1956, **39**, 463.
[22] Rüegg, Montavon, Ryser, Saucy, Schwieter, and Isler, *Helv. Chim. Acta*, 1959, **42**, 854.