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MAJOR CAROTENOIDS OF *SHEPHERDIA CANADENSIS*. ISOLATION AND SYNTHESIS OF METHYL APO-6'-LYCOPENOATE*

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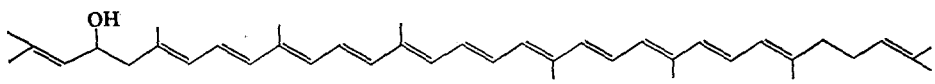
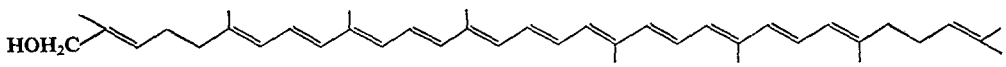
Abstract—Lycopene (III) and methyl apo-6'-lycopenoate (IV) are the major carotenoids of ripe berries of *Shepherdia canadensis*. The structural assignment of the new apo-carotenoid (IV) followed from its spectroscopic properties including NMR and mass spectra as well as the chemical and physical properties of various derivatives (V–IX), and was confirmed by synthesis via apo-8'-lycopenal (XII) and carbomethoxymethylene-triphenyl phosphorane (XIV).

INTRODUCTION

THE STRUCTURE of lycoxanthin, previously considered to be (I),[1] has recently been revised to (II).[2,3]



(I)



(II)

In addition to the original source,[1] lycoxanthin has been claimed to be present in various other organisms; for a review see Ref. 2. Since it was of interest to check if I was a naturally occurring carotenoid, a reinvestigation was carried out of a source in which lycoxanthin acetate was stated to be present.[4]

RESULTS AND DISCUSSION

Ripe berries of *Shepherdia canadensis* (L.) Nutt. contained 0·97 per cent carotenoids of the dry weight. Lycopene (III) constituted *ca.* 42 per cent of the total carotenoid and was identified by direct comparison with authentic lycopene, including co-chromatography tests of the iodine-catalysed equilibrium mixtures, mixed m.p. and visible and i.r. spectra.

* Part III in the series "Carotenoids of Higher Plants". For Part II see *Phytochem.* **8**, 185 (1969).
† Graduate Student, The Technical University of Norway, 1968.

[1] L. ZECHMEISTER and L. v. CHOLNOKY, *Ber.* **69**, 422 (1936).
[2] M. C. MARKHAM and S. LIAAEN-JENSEN, *Phytochem.* **7**, 839 (1968).
[3] L. CHOLNOKY, J. SZABOLCS and E. S. WAIGHT, *Tetrahedron Letters* 1931 (1968).
[4] A. STABURSVIK, *Acta Chem. Scand.* **8**, 1305 (1954).

Methyl apo-6′-lycopenoate (IV) constituted *ca.* 45 per cent of the total carotenoid. Its adsorptive properties and negative silylation indicated that hydroxy functions were absent. The visible spectrum (Fig. 1) indicated the presence of a conjugated carbonyl function. Hydride reduction provided a product (V) which according to its visible spectrum possessed an aliphatic decaene chromophore, and was a mono-ol, judged from its partition behaviour and the absence of detectable intermediates in the acetylation reaction (to VI). Allylic oxidation of the mono-ol (V) gave an oxidation product VII, differing in visible spectrum and adsorptive

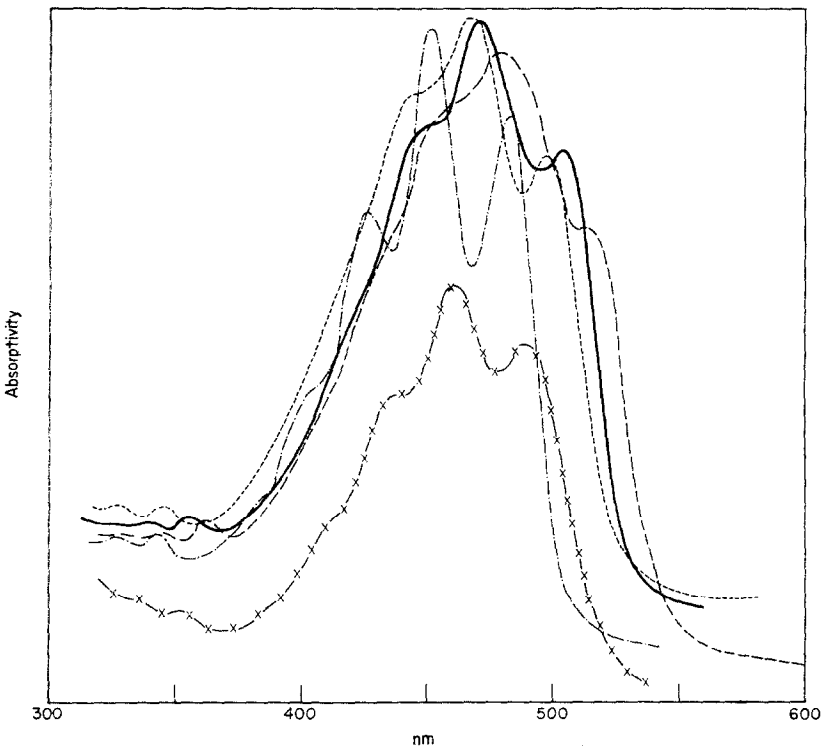


FIG. 1. VISIBLE LIGHT ABSORPTION SPECTRA IN PETROLEUM ETHER OF

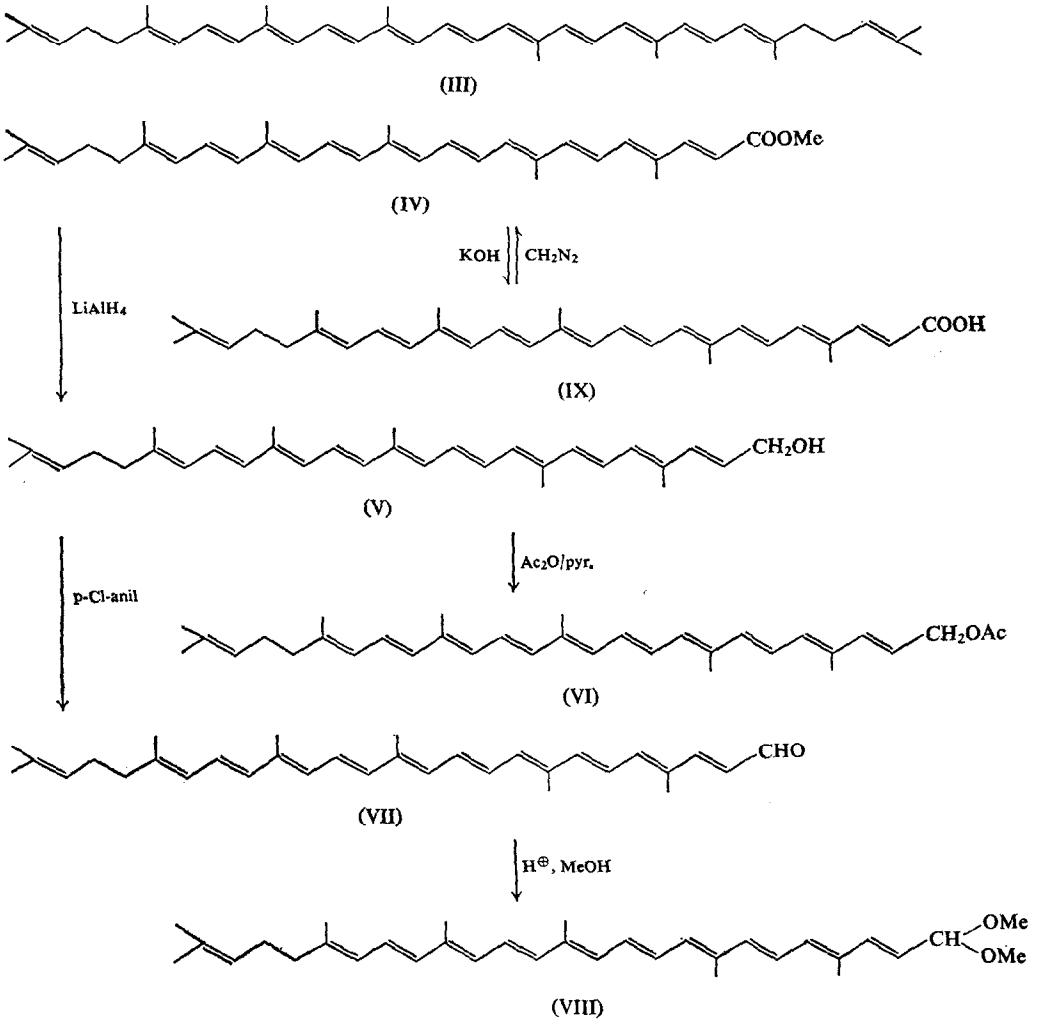
——— methyl apo-6′-lycopenoate (IV)
— · — apo-6′-lycopenol (V)
– – – – apo-6′-lycopenal (VII)
×—× apo-6′-lycopenoic acid (IX, in methanol)
------- apo-8′-lycopenal (XII).

properties from the natural compound (IV), thus revealing different carbonyl functions in IV and VII. The oxidation product VII reacted smoothly with acidified methanol to a decaene-type product (VIII), suggestive of acetal formation. Like esters of carotenoid acids,[5] the natural compound IV was resistant to mild alkali treatment, but hydrolysed to the corresponding acid (IX) under more forcing conditions. The carboxylic acid character of IX was supported by its partition ratio in neutral *contra* alkaline systems, adsorptive properties and maintenance of spectral fine-structure of the spectrum in methanol.[5] Moreover the acid (IX) was methylated with diazomethane to its methyl ester (IV), indistinguishable from the natural

[5] A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **19**, 1843 (1965).

ester in chromatographic properties and visible spectrum. This reaction sequence thus indicated the presence of an acyclic decaene system conjugated with a carbomethoxy group in the natural compound.

The i.r.-spectrum (Fig. 2) of IV is in agreement with the α,β-unsaturated methyl ester formulation (1705, 1195 and 1175 cm^{-1}), and complex absorption in the 960 cm^{-1} region suggested the presence of a neighbouring *trans* disubstituted double bond.[6] The NMR spectrum



(Fig. 3) had methyl signals at τ 6·24 (3 H; methyl ester), 8·03 (12 H; in-chain-methyl), 8·18 (3 H; end-of-chain methyl) and 8·32, 8·39 (3 + 3 H; isopropylidene). Allylic methylene signals at τ 7·70–7·91 integrated for 4 H. Two doublets at τ 2·61 and 4·12 (2 H, $J = 15{\cdot}4$ cps) were ascribed to olefinic protons on a double bond allylic to the carbonyl group, cf. Ref. 7, a multiplet centred at τ 4·85 corresponded to the olefinic isopropylidene proton[2] and signals

[6] H. KJØSEN and S. LIAAEN-JENSEN, unpublished results.
[7] M. S. BARBER, L. M. JACKMAN and B. C. L. WEEDON, *Proc. Chem. Soc.* 23 (1960).

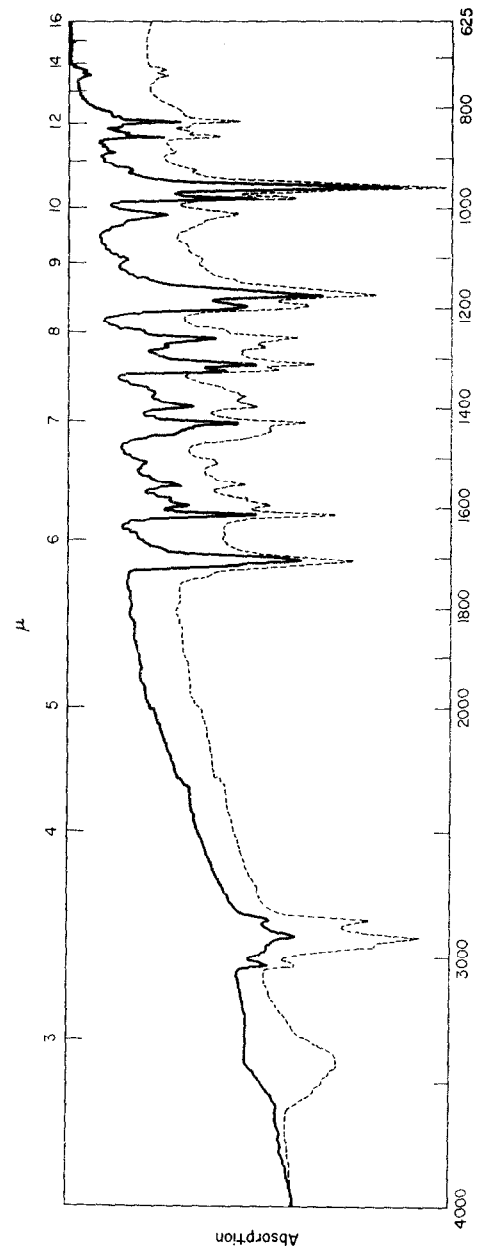


FIG. 2. INFRARED SPECTRA IN KBr OF METHYL APO-6′-LYCOPENOATE
—— natural, ------- synthetic.

in the τ 3·0–4·0 region to the olefinic protons of the polyene chain. The mass spectrum (Fig. 4) showed the molecular ion at *m/e* 472. Characteristic losses of 69 mass units from the molecular ion and the M-106 species and a moderate intensity peak at *m/e* 69 demonstrated a lycopene-type end-group.[8,9] A weak peak at M-59 agreed with the methyl ester formulation.[9]

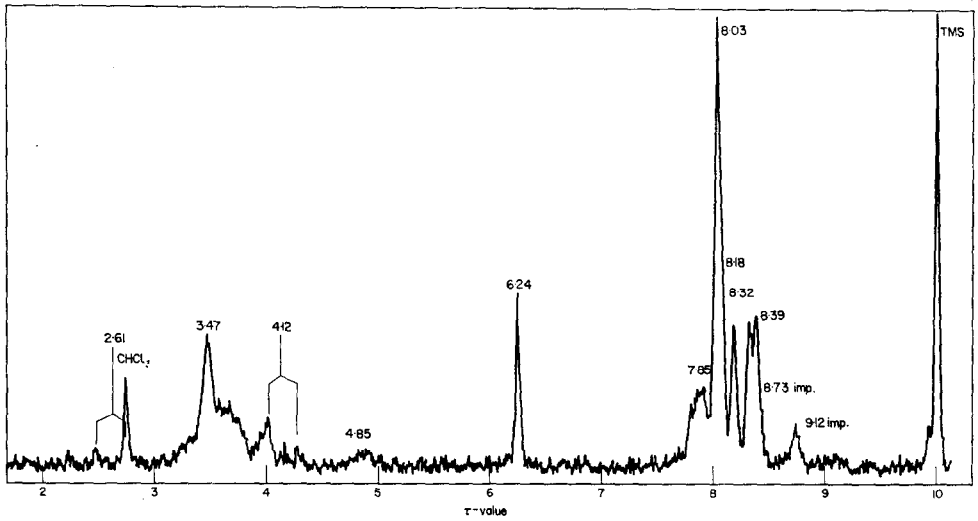
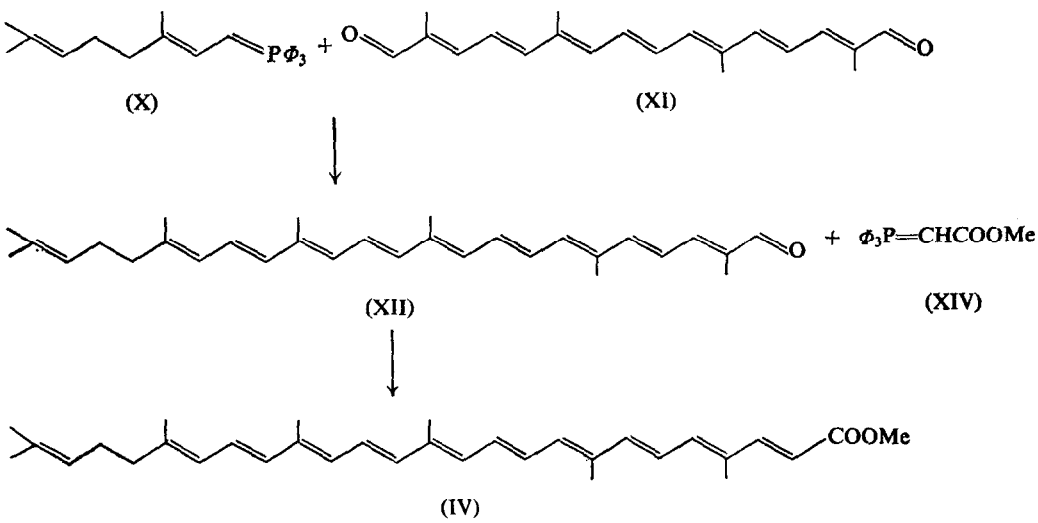


FIG. 3. PROTON MAGNETIC RESONANCE SPECTRUM IN DEUTEROCHLOROFORM OF METHYL APO-6′-LYCOPENOATE.



The above chemical and spectroscopic evidence was thus in complete agreement with structure IV, unequivocally proved by total synthesis. Geranylidenetriphenyl phosphorane

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

[9] C. R. ENZELL, G. W. FRANCIS and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **22**, in press (1968).

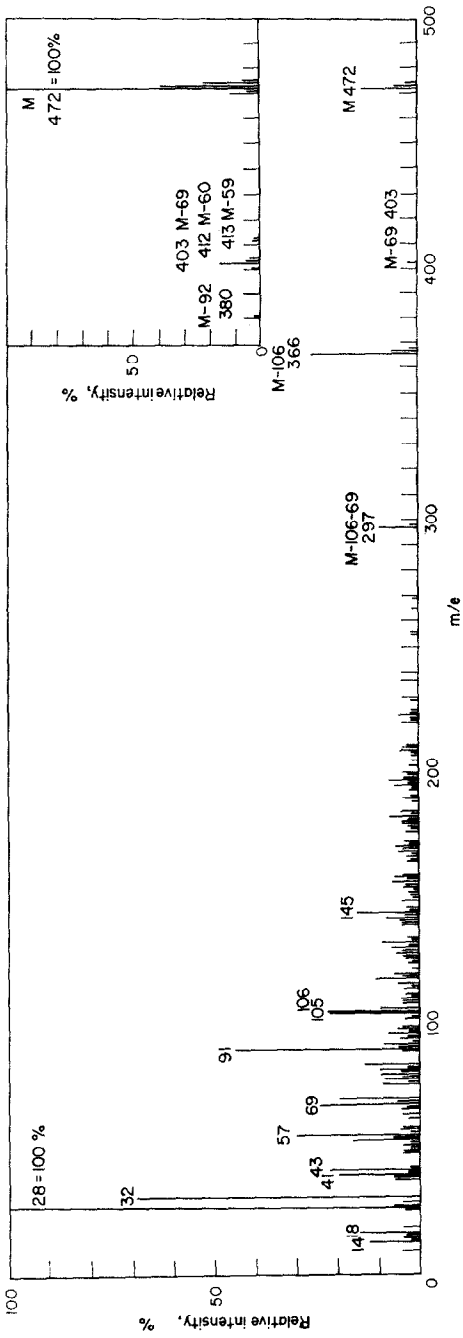


FIG. 4. MASS SPECTRUM OF METHYL APO-6'-LYCOPENOATE.

(X), prepared from linalool in the usual way,[10] was condensed with crocetindial (XI) in a Wittig reaction to the previously described apo-8′-lycopenal (C_{30}) (XII).[11] Apo-8′-lycopenal (XII) was in a second Wittig reaction condensed with carbomethoxymethylenetriphenyl phosphorane (XIV) prepared from methyl bromoacetate via its Wittig salt (XIII) in the usual manner.[12] The synthetic ester (IV) could not be chromatographically separated from the natural one (iodine-catalysed equilibrium mixtures), and the synthetic and natural esters gave no depression in mixed m.p. determination and exhibited identical visible, i.r. (Fig. 2) and NMR spectra. Separation of apo-8′-lycopenal (XII) and methyl apo-6′-lycopenoate (IV) could not be achieved on kieselguhr paper,[13] but TLC using kieselgel as adsorbent gave satisfactory separation (Table 1).

Judged by its relative abundance and melting point methyl apo-6′-lycopenoate (IV) is considered to be the compound previously identified as lycoxanthin acetate.[4] The corresponding acid (IX) derived from the natural ester in our hands melted at 174°.

Several minor carotenoids obtained from *Shepherdia canadensis* were not obtained in the pure state. Three resembled phytofluene, ζ-carotene and a bicyclic carotene.

TABLE 1. R_f-VALUES OF METHYL APO-6′-LYCOPENOATE AND RELATED COMPOUNDS

Trans compound	Kieselguhr paper[13] 2%*	5%	50%	Kieselgel plate (chloroform)
Apo-6′-lycopenal dimethyl acetal (VIII)	0·80			
Apo-8′-lycopenal (XII)	0·46	0·77		0·60
Methyl apo-6′-lycopenoate (IV)	0·46	0·77		0·74
Apo-6′-lycopenal (VII)	0·39	0·55		
Apo-6′-lycopenol (V)	0·34			
Apo-6′-lycopenoic acid (IX)			0·23	

* Acetone in petroleum ether.

Hitherto twenty apo-carotenoids, excluding vitamin A derivatives, have been isolated from natural sources. None of these have previously been encountered in the Eleagnaceae, the family to which *S. canadensis* belongs. The systematic position of Eleagnaceae and its three genera has been disputed.[14–18] However, it does not appear that the carotenoid distribution of *S. canadensis* gives any clue to its correct taxonomic position.

EXPERIMENTAL

Materials and Methods

When not specified these were as summarized elsewhere.[19] I.r.-spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer, NMR spectra on a Varian A 60 A instrument and mass spectra on an LKB-

[10] J. D. SURMATIS and A. OFNER, *J. Org. Chem.* **28**, 2735 (1963).
[11] R. BONNET, A. A. SPARK and B. C. L. WEEDON, *Acta Chem. Scand.* **18**, 1739 (1964).
[12] O. ISLER, H. GUTMANN, M. MONTAVON, R. RÜEGG, G. RYSER and P. ZELLER, *Helv. Chim. Acta* **40**, 1242 (1957).
[13] A. JENSEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **13**, 1863 (1959).
[14] A. ENGLER, *Syllabus der Pflanzenfamilien*, Vol. 2. Bortentraeger, Berlin (1964).
[15] J. HUTCHINSON, *The Families of Flowering Plants*, Vol. 2, 2nd Ed. Oxford University Press, Oxford (1959).
[16] A. CRONQVIST, *Bull. Jard. Bot. L'Etat, Bruxelles* **27**, 13 (1957).
[17] A. TAKHTAJAN, *Die Evolution der Angiospermen*, Gustav Fischer, Jena (1959).
[18] G. ZERVETTAZ, Monographie der Eleagnacees, *Beih. Bot. Zbl.* **2**, 1 (1909).
[19] A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **20**, 1970 (1966).

9000 mass spectrometer with direct inlet system at 70 eV with an ion source temperature of 290°. TLC was performed on kieselgel G. R_f-values of derivatives of IV are given in Table 1.

Shepherdia canadensis

Biological material. Ripe berries of *Shepherdia canadensis* (L.) Nutt. (390 g) were collected in September 1967 in Eklutna Valley, Chugach Mountains, Alaska, and stored frozen.

Extraction. The frozen berries were thawed, ground in a mortar, dehydrated with methanol and extracted with acetone in the presence of solid CO_2 in a Waring blendor. The pigments were transferred to ether–petroleum ether on dilution with aqueous NaCl. The combined ether extract was washed repeatedly with aqueous NaCl to remove saponins and dried: yield 330 mg total carotenoid or 0·97 per cent of the extracted residue.

Saponification. Standard saponification (5% methanolic KOH, 1 hr) of a sample revealed no changes in the carotenoid composition on kieselguhr paper. 175 mg carotenoids were therefore purified in this manner; pigment recovery was 93 per cent.

Column chromatography. Unless otherwise specified, column chromatography was carried out on neutral alumina activity grade 2.

Minor Carotenoids

Carotenoids of the non-polar fractions were further separated on alumina paper (petroleum ether) providing a fluorescent phytofluene-like compound (R_f 0·66; λ_{max} 330, 347 and 365 nm in petroleum ether), a ζ-carotene-like compound (R_f 0·65; λ_{max} 377, 398 and 423 nm), and a bicyclic carotene-like compound (R_f 0·54; λ_{max} 425, 448 and 474 nm). No attempt was made to characterize the more polar minor carotenoids.

Lycopene (III)

From the alumina column 5–10% ether was required for elution. After crystallization from benzene/methanol the yield was 8·5 mg, m.p. 155–160°; synthetic lycopene had m.p. 174–175° and the mixed m.p. was at 166–173°. The visible spectrum in petroleum ether (at λ_{max} $E^{1\%}_{1cm}=2840$; purity 82 per cent) corresponded to that of synthetic lycopene. The i.r. spectrum (KBr, ν_{max} 3040, 2970–2850, 1630, 1550, 1440, 1395–1365, 960 and 830 cm^{-1}) was identical with that of synthetic lycopene. The iodine catalysed equilibrium mixture in petroleum ether of natural III separated on kieselguhr paper (petroleum ether) contained *trans* (51 per cent of total, R_f 0·48; λ_{max} 446, 472 and 504 nm in acetone), neo A (32 per cent of total, R_f 0·63; λ_{max} 443, 469 and 500 nm) and neo B (17 per cent of total, R_f 0·77; λ_{max} 440, 465 and 493 nm) isomers, which agreed in relative abundance, λ_{max} and R_f values with those of synthetic lycopene.

Methyl Apo-6′-Lycopenoate (IV)

For elution from alumina 15–20% ether in petroleum ether was required. Crystallization and recrystallization from benzene–methanol provided 10·3 mg, m.p. 137–145°, λ_{max} (448), 471 ($E^{1\%}_{1cm}=2600$) and 503 nm in petroluem ether (Fig. 1) and 445, 469 and (495) nm in acetone. The i.r.-spectrum in KBr pellet is given in Fig. 2 and the NMR spectrum in $CDCl_3$ in Fig. 3. The mass spectrum (Fig. 4) had characteristic peaks at *m/e* 472 (M), M-59, M-69, M-92, M-106 and M-106-69. Iodine-catalysed isomerization in petroleum ether caused the formation of a neo A isomer (32 per cent of total, R_f 0·62; λ_{max} 341, 357, (443), 465 and 495 nm) and a neo B isomer (14 per cent of total, R_f 0·47, λ_{max} 341, 357, (443), 465 and 494 nm). R_f values refer to kieselguhr paper (2% acetone in petroleum ether). Silylation of IV (0·1 mg) in the usual manner gave 100 per cent pigment recovery. No new products were formed.

Apo-6′-lycopenol (V). Natural IV (1·0 mg) in dry ether was reduced with $LiAlH_4$; pigment recovery was 52 per cent. Chromatographed on alumina (activity grade 3), V required 15% ether in petroleum ether for elution, had λ_{max} 426, 451 and 483 nm in petroleum ether (Fig. 1) and partition ratio 52:48 in petroleum ether/95% methanol.

Apo-6′-lycopenol acetate (VI). V (0·1 mg) was acetylated for 1 hr. No intermediates were detected by paper chromatography of samples taken during the reaction. The visible spectrum of VI corresponded to that of V.

Apo-6′-lycopenal (VII). V (0·25 mg) was oxidized with *p*-chloranil; pigment recovery was 94 per cent. The aldehyde (VII) was purified by column chromatography; yield 0·13 mg, λ_{max} in petroleum ether (462), 479 and (515) nm (Fig. 1), in acetone (448), 473 nm, and in methanol 477 nm.

Apo-6′-lycopenal dimethyl acetal (VIII). VII (0·12 mg) was treated with acidified methanol.[20] Paper chromatography revealed smooth formation of yellow VIII, λ_{max} 425, 447 and 485 nm in petroleum ether for neo A. *Trans* VIII was not readily separated from *cis* VII.

Apo-6′-lycopenoic acid (IX). IV (10·2 mg) in ether (1 ml) was treated with 10% methanolic KOH (50 ml) for 24 hr at room temperature. Neutral pigments were transferred to ether and hypophasic (acidic) pigments transferred to ether after acidification with acetic acid; yield 5·9 mg IX, further purified by chromatography

[20] A. J. AASEN and S. LIAAEN-JENSEN, *Acta Chem. Scand.* **21**, 2185 (1967).

on cellulose and crystallized from $CHCl_3$–petroleum ether, m.p. 174°, λ_{max} (447), 468, and (493) nm in acetone and (440), 460, and 488 nm in methanol (Fig. 1), ν_{max} (KBr) 3400, 3030, 2910–2850, 1680, 1610–1510, 1430, 1380, 1315, 1280, 980–960, 860, 830, and 720 cm^{-1}. IX could not be eluted with 100% acetone from alumina paper.

The acid (IX) was methylated with diazomethane in ether to a product indistinguishable from the natural compound (IV) in visible spectrum and adsorptive behaviour.

Synthesis of Methyl Apo-6′-lycopenoate (IV)

Geranylidenetriphenyl phosphorane (X). Linalool was converted to geranyl triphenylphosphonium bromide, m.p. 189°, according to the procedure of Surmatis and Ofner.[10] From this salt (810 mg) X was prepared according to the procedure of Bonnet *et al.*[11] using butyl lithium.

Apo-8′-lycopenal (XII) was prepared as described by Bonnet *et al.*[11] from crocetindial (XI, 500 mg); yield lycopene (III, 198 mg), apo-8′-lycopenal (XII), 195 mg) and recovered crocetindial (XI, 207 mg). XII was purified by column chromatography (eluent 10–20 per cent ether in petroleum ether) and crystallized from benzene-methanol, yield 135 mg, m.p. 138–140°, after recrystallization twice (Bonnet *et al.*[11] state m.p. 139–140°), λ_{max} 468 nm (petroleum ether; Fig. 1), 470 nm (acetone or methanol), ν_{max} (chloroform) 3035, 2980–2850, 2720, 1660, 1610–1510, 1440–1405, 1380–1360, 1270, 1181, 1000, 968, and (825) cm^{-1}, τ 8·39, 8·32 (3+3 H, isopropylidene), 8·19 (3 H, end-of-chain methyl), 8·02 (12 H, in-chain methyl), 7·85 (4 H, allylic methylene), 4·86 (1 H, isopropylidene), 3·15–3·95 (*ca.* 14 H, olefinic), and 0·51 (1 H, aldehydic).

Carbomethoxymethylenetriphenyl phosphorane (XIV). Methyl bromoacetate was prepared by methylation of bromoacetic acid with diazomethane,[21,22] and carbomethoxymethyltriphenylphosphonium bromide (XIII) was prepared according to Isler *et al.*[12] from methyl bromoacetate (10·6 g) and triphenylphosphine; yield 23 g (77 per cent), m.p. 165–166°, Isler *et al.* give m.p. 163°. XIV was prepared from the above salt (23 g);[12] yield 7·4 g (38 per cent), m.p. 170–171°, λ_{max} 222 and 268 nm in ethanol, τ 2·24, 2·48 (15 H), 2·56 (1 H), and 6·48 (3 H). Isler *et al.* report m.p. 162–163°, λ_{max} 222 and 268 nm in ethanol.

Methyl apo-6′-lycopenoate (IV) was prepared by a method analogous to that described by Isler *et al.*[12] for methyl bixin, XII (90 mg) and XIV (65 mg) in dry benzene (50 ml) were refluxed for 40 hr with addition of further XIV (65+130 mg) after 4 and 30 hr respectively; pigment recovery was 100 mg after column chromatography. Re-chromatography and crystallization from benzene–methanol yielded 26 mg IV, m.p. 141–144°, λ_{max} in petroleum ether (448), 471 ($E^{1\%}_{1cm}$=2270) and 503 nm and in acetone 445, 469 and (495) nm, ν_{max} 3040, 2980–2850, 1705, 1620–1500, 1430, 1400–1360, 1195, 1175, 980, 960 and 830 cm^{-1} (Fig. 2), τ 8·39, 8·32 (3+3 H), 8·18 (3 H), 8·03 (12 H), 7·70–7·90 (4 H), 6·24 (3 H), 4·85 (1 H), 2·61 and 4·12 (J=15·4 cps) and 3·0–4·0 (*ca.* 15 H).

Synthetic and natural *trans* IV could not be separated on a kieselgel plate. The quantitative composition (relative abundance, electronic spectra and R_f values on kieselguhr paper of *trans*, neo A and neo B) agreed completely with that of natural IV. Mixed m.p. determination gave no depression.

Acknowledgements—We are indebted to Professor N. A. Sørensen for suggesting this investigation and for his inspiring interest in the work. The biological material was kindly provided by W. Mitchell, Alaska Agricultural Experiment Station, University of Alaska, Palmer. Crocetindial was a gift from Dr. J. D. Surmatis, Hoffmann—La Roche, Nutley, New Jersey, U.S.A. The mass spectrum was recorded by the courtesy of Dr. C. Enzell, Swedish Tobacco Co., Stockholm. Dr. M. Kelly kindly made linguistic corrections to the manuscript.

[21] H. v. PECHMANN, *Ber.* **28**, 855 (1895).
[22] J. T. DEBOER and H. J. BACKER, *Receuil* **73**, 229 (1954).