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Bacterial Carotenoids, XLVI.* C_{50}-Carotenoids, 14.**

C_{50}-Carotenoids from *Arthrobacter glacialis*

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From the psychrophilic bacterium *Arthrobacter glacialis* have been isolated three C_{50}-carotenoids with molecular formulae $C_{50}H_{72}O_2$: the bicyclic decaprenoxanthin (*1a*, 7 % of total carotenoids), the aliphatic bisanhydrobacterioruberin (*2a*, 10 %) and the monocyclic *A.g.* 470 (*3a*, 83 %).

Decaprenoxanthin (*1*) and bisanhydrobacterioruberin (*2*) were in all respects, including chiroptical properties, identical with known carotenoids.

The constitution of the previously undescribed *A.g.* 470 (*3a*) followed from its spectral properties (electronic, ^{1}H NMR including Eu-shift experiments and mass spectra) and derivatization to *3b* and *3c*. *3a* suffered remarkable elimination to the tridecaene *4a* ($C_{47}H_{64}O$) upon DMSO/KOMe/MeOH treatment. Judged by CD data *A.g.* 470 (*3a*) also in stereochemical respect *3a* appears to be half decaprenoxanthin (*1a*) + half bisanhydrobacterioruberin (*2a*).

The intensity ratios of the M − 92/M − 106 ions on electron impact of *3a,b,c* and *4a,b* are consistent with the general theory.

So far altogether fifteen different C_{50}-carotenoids have been encountered in Nature.[1–4] They may all be considered as 2,2′-isopentenylated C_{40}-carotenoids and represent bicyclic or monocyclic modifications with β- or ε-rings [5] and aliphatic carotenoids of varying chromophore. The oxygen functions are restricted to primary allylic or tertiary alcohols and glycosides thereof.[1–4]

Their known occurrence indicates that C_{50}-carotenoids may be restricted to spheric or rod-shaped, aerobic, gram-positive, non-photosynthetic bacteria.[1–4,6]

We now report the isolation of two known and a major, previously undescribed monocyclic C_{50}-diol with ε-ring from a psychrophilic bacterium. Its structure and occurrence fit into the general pattern described above.

RESULTS AND DISCUSSION

Arthrobacter glacialis Moiroud and Gounot is a yellow, gram-negative (or gram-variable), polymorph, psychrophilic bacterium of the family Corynebacteriaceae, isolated from morainic mud.[7] The pigmentation is due to carotenoids, previously subjected to preliminary studies.[8,9]

In the present work *A. glacialis*, cultivated on 6500 Petri dishes in a glucose/trypton enriched medium at 6 °C, contained 78 mg total carotenoid.

Colourless lipids were removed by repeated precipitation from acetone. Column chromatography followed by TLC on silica gel provided decaprenoxanthin (*1a*, 4.5 mg, 7 % of total), bisanhydrobacterioruberin (*2a*, 6.5 mg, 10 % of total) and *A.g.* 470 (*3a*, 55 mg, 83 % of total).

Decaprenoxanthin (*1a*) was identified on the basis of its electronic spectrum, mass spectrum (*m/e* 704 = M, consistent with $C_{50}H_{72}O_2$, M − 18, M − 92, M − 106, M − 140 (RDA-fragmentation)), co-chromatography with *1a ex Flavobacterium*

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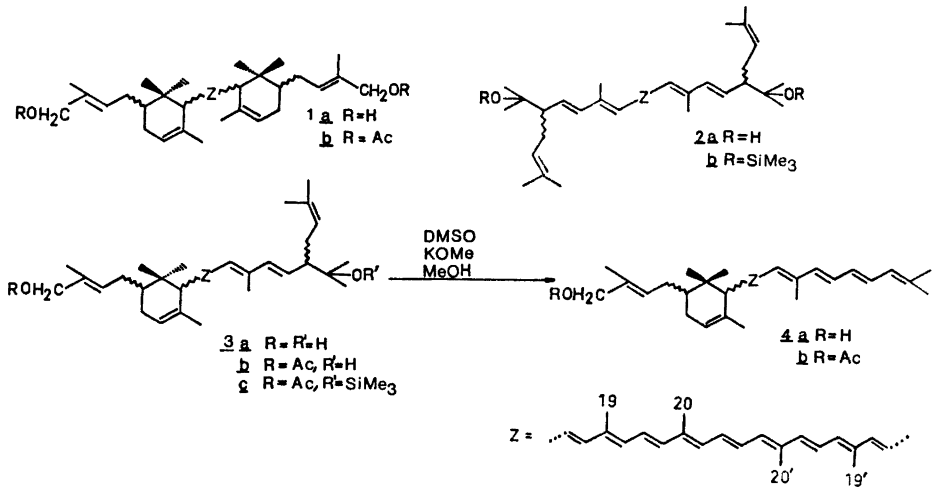


dehydrogenans [10] and transformation to the diacetate *1b* (*m/e* 788=M, compatible with $C_{50}H_{70}(OCOCH_3)_2$, M−60, M−92, M−106, M−182 (RDA-fragmentation)). The CD spectrum of *1b* was fully consistent with that of decaprenoxanthin (*1a*) *ex F. dehydrogenans*,[11] demonstrating the same, so far unestablished, chirality of decaprenoxanthin from the two sources.

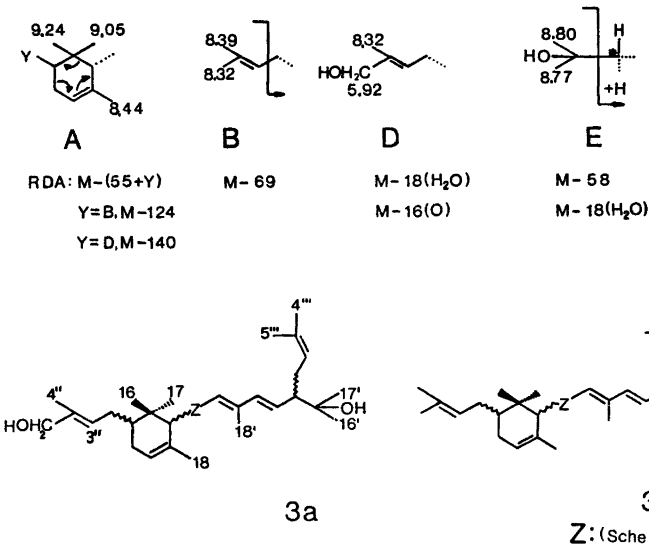
Bisanhydrobacterioruberin (*2a*) was identified by the following criteria. Its electronic spectrum and mass spectrum (*m/e* 704=M, compatible with $C_{50}H_{72}O_2$, M−18, M−18−18, M−58, M−69, M−92, M−106) corresponded to those of *2a ex Corynebacterium poinsettiae*.[12] Cochromatography gave no separation. Upon silylation *2a* gave a di(trimethylsilyl) ether (*m/e* 848=M, corresponding to $C_{50}H_{70}(OSi(CH_3)_3)_2$, M−15, M−58, M−72, M−92, M−106, 131). Finally the CD spectrum of *2a* corresponded to that of bisanhydrobacterioruberin (*2a*) *ex C. poinsettiae* [11] demonstrating the same, unknown, chirality at C-2,2′ for bisanhydrobacterioruberin from the two sources.

A.g. 470 (*3a*) exhibited electronic spectra characteristic of an aliphatic undecaene chromophore. Its polarity (Table 1) was indicative of a C_{50}-diol. Acetylation resulted in a monoacetate *3b* and silylation of the monoacetate gave a monoacetate-mono(trimethylsilyl) ether *3c*, demonstrating the presence of one primary or secondary and one tertiary hydroxy group. The diol assignment was supported by the mass spectra of *3a* (*m/e* 704=M, compatible

Table 1. Adsorptive properties of the carotenoids from *A. glacialis* and their derivatives.

Carotenoid	Schleicher & Schüll 287 kieselguhr paper				
	0 %[a]	1 %	2 %	5 %	10 %
Decaprenoxanthin (*1a*)	0		0.06	0.46	0.65
diacetate (*1b*)	0.13	0.46	0.74		
A.g. 470 (*3a*)	0			0.21	0.60
monoacetate (*3b*)			0.13	0.66	
monoacetate mono TMS ether (*3c*)			0.88		
Bisanhydrobacterioruberin (*2a*)					0.51
di TMS ether (*2b*)	0.81				

[a] Acetone in petroleum ether.



with $C_{50}H_{72}O_2$), *3b* (*m/e* 746=M; $C_{50}H_{70}$-$(OCOCH_3)OH$) and *3c* (*m/e* 818=M; $C_{50}H_{70}$-$(OCOCH_3)OSi(CH_3)_3$). Molecular ions were confirmed by fragment ions (M−92, M−106, M−158) associated with losses from the polyene chain.[13] Characteristic fragment ions attributed to the end groups were observed for the diol *3a* (M−18, M−76=M−18−58?, M−124=M−106−18?, M−140=M−16−124?, M−142=M−18−124?) and the monoacetate *3b* (M−18, M−42, M−58, M−60, M−78=M−18−60?, *m/e* 622.474=M−124=M−C_8H_{10}−H_2O, M−142=M−18−124?, M−164=M−106−56?, M−182).

^{1}H NMR data ($CDCl_3$) of the free diol showed the presence of four in-chain methyl groups (δ 1.98, *ca.* 12 H) and one near-end-of-chain methyl group (δ 1.93, 3 H), defining the chromophore. Methyl signals typical of an ε-end group A, Scheme 2: δ 0.76 (3 H), 0.95 (3 H) and 1.56 (3 H), of an isopropylidene end group B: δ 1.62 (3 H) and 1.68 (3 H), of a hydroxylated isopropylidene group D: δ 1.68 (3 H) and 4.02 (2 H) and of end group E: δ 1.20 (3 H), 1.23 (3 H) were further found for *3a*. Corresponding signals were observed for the monoacetate *3b* with the expected downfield shift of the acetylated end group D signal at δ 4.02 (2 H) to δ 4.46 (2 H).

However, unequivocal differentiation between *3a* and *3d* (Scheme 2) for *A.g.* 470 could not be made from these ^{1}H NMR and mass spectra. Thus fragment ions which may be attributed to RDA-fragmentation of *3d* (M−124) and *3a* (M−140) could also represent combination losses (M−124=106−18? and M−140=124−16?) from other functionalities present. For the monoacetate *3b* accurate mass measurements demonstrated that the M−124 ion indeed represented such a combined loss (M−toluene−H_2O) rather than a one-step loss of a hydrocarbon fragment.

This ambiguity with the two hydroxy functions located at the same or opposite extremity of the molecule readily lends itself to analysis by the ^{1}H NMR Eu-shift technique.[14,15a] Stepwise addition of Eu(dpm)$_3$ to *A.g.* 470 demonstrated (Fig. 1) induced shifts of the 16,17 *gem.* dimethyl groups under conditions where no induced shift occurred for signals from the C-18,18′ range of the molecule, in preference of *3a*. As expected the highest induced shifts were connected with the protons of D and E (Scheme 2, Fig. 1) for *A.g.* 470 (*3a*). *trans*-Configuration of the $\Delta 3''$-bond is assumed on the basis of identical chemical shift of the $-CH_2OH$ signal (δ 4.02) in *A.g.* 470 (*3a*) and decaprenoxanthin (*1a*), for which *trans*-configuration has been demonstrated.[15b]

The (M−92)/(M−106) intensity ratio observed in the mass spectrum of *A.g.* 470 (*3a*) is within the range predicted[16,17] for this chromophore with bulky C-2′ substituents. Also the magnetic non-equivalence of the two

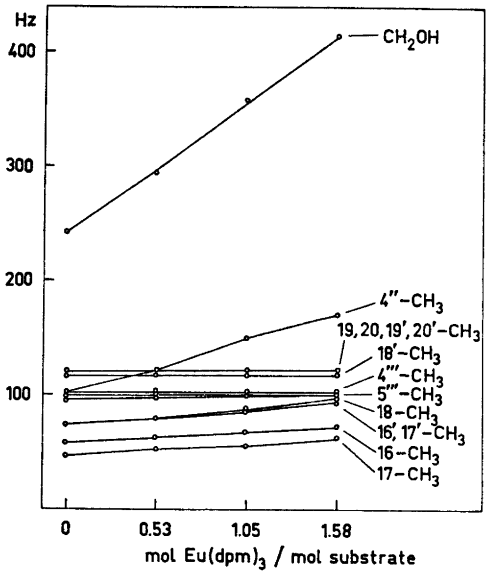


Fig. 1. Induced chemical shifts (Hz) on stepwise addition of Eu(dpm)$_3$ to *A.g.* 470 (*3a*) in $CDCl_3$.

gem. dimethyl groups 16',17' supports the branching point at C-2'.[13] Location of the second extra C_5-unit to C-2 mainly rests on the RDA fragmentation on electron impact (M − 140 for *3a*, M − 182 for *3b*).

Formally [5] *A.g.* 470 (*3a*) is 2-(4''-hydroxy-3''-methyl-2''-butenyl), 2'-(3'''-methyl-2'''-butenyl)-3',4'-didehydro-1',2'-dihydro-ε,ψ-carotene-1'-ol, and in principle *3a* = half *1a* + half *2a*.

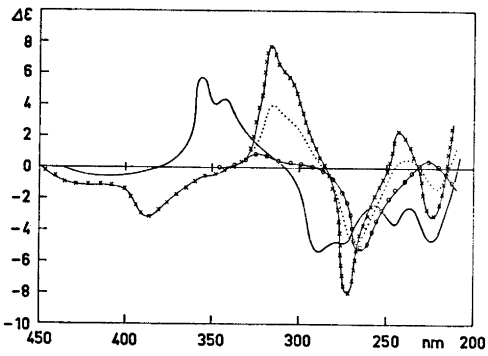


Fig. 2. CD spectra in EPA (diethyl ether-isopentane-ethanol 5:5:2) solution of carotenoids *ex A. glacialis:* O decaprenoxanthin diacetate (*1a*) × bisanhydrobacterioruberin (*2a*), ——— *A.g.* 470 (*3a*) obs., ··· *A.g.* 470 (*3a*) calc. from half CD *1a* + half CD *2b*.

If, as expected from biosynthetic considerations, the ε-end group of *3a* possessed the same chirality at C-2 and C-6 as *1a* and the aliphatic end group the same chirality as the corresponding end group of *2a*, the CD-curve of *A.g.* 470 (*3a*) should be half CD of *1a* + half CD of *2a*, provided the additivity rule of Klyne and co-workers [18] for ORD spectra of bicyclic carotenoids could be expanded to CD spectra of monocyclic carotenoids. So far the additivity rule has proved valid for CD spectra of various bicyclic carotenoids.[19,20] Fig. 2 gives the CD spectra of decaprenoxanthin (*1a*), bisanhydrobacterioruberin (*2a*) and *A.g.* 470 (*3a*), all *ex. A. glacialis*, and of *3a* calculated on the basis of the additivity hypothesis. Only a rough agreement (negative Cotton effect below *ca.* 290 nm and positive Cotton effect above) is found for the observed and calculated values for *3a*. The 20 − 40 nm bathochromic displacement of the observed CD spectrum relative to the calculated one may reflect chromophoric differences, a phenomenon not yet well understood.

In an attempt to isomerize the ε-end group to a β-end group by the procedure of Andrewes [21] for stereochemical studies, a remarkable elimination was observed. Thus treatment of the diol *3a* with KOMe/MeOH/DMSO provided in 15 % yield *4a* with aliphatic tridecaene chromophore; *m/e* 644 = M, corresponding to $C_{47}H_{64}O$; M − 92, M − 106. Upon acetylation *4a* gave a monoacetate *4b* (*m/e* 686 = M, corresponding to $C_{47}H_{63}OCOCH_3$; M − 60, M − 92, M − 106). *4b* failed to give a trimethylsilyl ether on silylation, confirming that the tertiary hydroxy function was lost during the elimination. Two-three times increase in the M − 92/M − 106 intensity ratios on electron impact of *4a, b* relative to *3a,b* is consistent with the general hypothesis.[16,17,22] Thus one extra mode of xylene elimination is introduced when the steric hindrance from the C − 2' substituent is removed. However, this may be counteracted by two extra modes of toluene elimination by prolongation of the chromophore.

Attempts to prepare the analogous pentadecaene di-elimination product from bisanhydrobacterioruberin (*2a*) failed, since *2a* decomposed under the reaction conditions employed.

EXPERIMENTAL

Biological material. Arthrobacter glacialis Moiroud and Gounot [7] was grown in 6500 Petri dishes at 6 °C for 2 months at pH 7 on a solid medium [23] containing: trypticase 5 g, liver extract 2.5 g, K_2HPO_4 1 g, $MgSO_4$ 0.1 g, $CaCl_2$ 0.05 g, glucose 5 g, trace element solution 0.1 ml (containing $ZnSO_4$ 0.02 g, $MnSO_4$ 0.02 g, H_2BO_3 0.5 g, Na_2MoO_4 0.5 g, $CoCl_2$ 0.02 g, and $FeCl_3.6\ H_2O$ 3 ml per 100 ml) and H_2O to 1000 ml.

Other materials and methods were as commonly employed in the Norwegian laboratory.[24] CD spectra were recorded in EPA solution on a Roussel-Jouan dicrographe. Acetylation and silylation were effected by standard methods.[25] Adsorptive properties of the pigments studied are compiled in Table 1.

Isolation of the carotenoids. The cells were removed from the agar surface by means of the blade of a knife and extracted with acetone at room temperature: estimated yield 78 mg (E(1 %, 1 cm) = 2500 at λ_{max}). Colourless lipids were removed by five consecutive precipitations from a concentrated acetone solution; estimated lipid content > 20 (carotenoid content). Since column chromatography on deactivated alumina gave unsatisfactory pigment recovery, chromatography on the weaker, but less selective silica gel columns, was resorted to. Due to additional lipids some carotenoids were eluted with benzene – 30 % chloroform in benzene. However, 96 % of the total carotenoids were eluted as a mixed fraction with 40 % chloroform in benzene. The mixture contained decaprenoxanthin (*1a*, *ca.* 4.5 mg; 7 % of total), *A.g.* 470 (*3a*, *ca.* 55 mg; 83 % of total) and bisanhydrobacterioruberin (*2a*, *ca.* 6.5 mg; 10 % of total) judged by TLC data below. Further lipids were removed by three successive TLC purifications on silica gel (developer 50 % ether in petroleum ether) also effecting quantitative separation of *1a*, *2a*, and *3a*.

Decaprenoxanthin (*1a*)

Decaprenoxanthin (1a), available *ca.* 3 mg had λ_{max} (acetone) 417, *441* and 470.5 nm, % III/II [26] = 98. Main maxima are given in italics. The mass spectrum had *m/e* 704 = M, M – 18, M – 79, M – 92, M – 106, M – 140. No separation was observed on co-chromatography with decaprenoxanthin *ex Flavobacterium dehydrogenans*,[10] *cf.* Table 1.

Decaprenoxanthin diacetate (1b), prepared by acetylation of *1a*, yield *ca.* 1 mg, had *m/e* 788 (M), M – 92, M – 106, M – 82, % (M – 92)/M – 106 [24,25] = 3.1. The CD spectrum is given in Fig. 2.

Bisanhydrobacterioruberin (*2a*)

Bisanhydrobacterioruberin (2a), available *ca.* 4 mg had λ_{max} (hexane) 370, 388, 465, *494*, 527, % III/II = 61; *m/e* 704 = M, M – 18, M – 18 – 18, M – 58, M – 69, M – 92, M – 106), 69 (base peak); % (M – 92)/(M – 106) = 0.13. The CD spectrum is given in Fig. 2. Co-chromatography with *2a ex Corynebacterium poinsettiae* [12] gave one zone, *cf.* Table 1). *2a* gave no acetylated product under standard acetylation conditions. *2a* was destroyed under the conditions [21] where *3a* was converted to *4a* below.

Bisanhydrobacterioruberin di(trimethylsilyl) ether (2b) had *m/e* 848 = M, M – 15, M – 58, M – 72, M – 92, M – 106, 131 (base peak).

A. g. 470 (*3a*)

A.g. 470 (*3a*), available *ca.* 40 mg containing some colourless lipid impurities, had λ_{max} (hexane) 345, 362, (420), 443, *470* and 502 nm, % III/II = 77; *m/e* 704 = M, M – 2, M – 18, M – 76, M – 92, M – 106, M – 128, M – 140, M – 142, M – 158, % (M – 92)/M – 106 = 0.35; δ ($CDCl_3$) 0.76 (3 H), 0.95 (3 H), 1.20 (3 H), 1.23 (3 H), 1.56 (3 H), 1.62 (3 H), 1.68 (6 H), 1.93 (3 H), 1.98 (*ca.* 12 H), 4.02 (2 H), 5.2 – 5.7 (3 H) and 6.0 – 6.7 (*ca.* 17 H). Stepwise addition of 2 mg $Eu(dpm)_3$ to final concentrations of 2, 4, and 6 mg $Eu(dpm)_3$/8 mg *3a* in $CDCl_3$ (0.4 ml) caused induced shifts as presented in Fig. 1. The CD spectrum of *3a* is given in Fig. 2.

A.g. 470 monoacetate (3b), prepared by standard acetylation [25] of *3a*, available *ca.* 7 mg not lipid-free, m.p. 85 °C (evacuated tube) exhibited electronic spectrum as *3a* with E (1 %, 1 cm) = 2065 at 470 nm in hexane (ε = 112 000); *m/e* 746 (M), M – 18, M – 58, M – 60, M – 78, M – 87, M – 92, M – 106, M – 118, M – 142, M – 150, M – 158, M – 164, % M – 92/106 = 0.27; δ ($CDCl_3$) 0.75 (3 H), 0.93 (3 H), 1.20 (6 H), 1.55 (3 H), 1.62 (3 H), 1.67 (6 H), 1.92 (3 H), 1.97 (*ca.* 12 H), 2.05 (*ca.* 4 H), 4.46 (2 H), 5.3 – 5.7 (3 H) and 6.0 – 7.0 (*ca.* 17 H).

A.g. 470 monoacetate mono(trimethylsilyl) ether (3c), prepared by standard silylation [25] of *3b* had electronic spectrum as *3a*, *m/e* 818 (M), M – 15, M – 58, M – 60, M – 92, M – 106, M – 124, M – 131, M – 140, M – 142, 131 (base peak), % M – 92/M – 106 = 0.29.

Elimination product 4a. 3a (1 mg) in DMSO (2 ml), benzene (0.5 ml), methanol (0.5 ml, containing 4.5 % KOMe) was kept under nitrogen at 110 °C for 15 min.[21] After cooling, methanol (2.5 ml) was added and the pigments extracted with ether in the usual manner. No *3a* was recovered. Upon TLC a purple product (*4a*, yield *ca.* 15 %), less polar than *3a*, was isolated. *4a* had λ_{max} (acetone) (370) 389, 468, *494*, 528 nm, % III/II = 44; *m/e* 644 (M), M – 92, M – 106, % M – 92/M – 106 = 0.72.

Acetylated elimination product 4b. 4b, prepared

from *4a* had electronic spectrum as *4a* and *m/e* 686 (M), M−60, M−84, M−92, M−106, % M−92/M−106 = 0.66. *4b* gave no trimethylsilyl ether on silylation.

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REFERENCES

1. Straub, O. In Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, Chapter XII.
2. Arpin, N., Liaaen-Jensen, S. and Trouilloud, M, *Acta Chem. Scand. 26* (1972) 2524.
3. Arpin, N., Fiasson, J.-L. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 2526.
4. Arpin, N., Norgård, S., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand. 27* (1973) 2321.
5. IUPAC Tentative rules for the nomenclature of carotenoids *Biochem. J. 127* (1972) 741.
6. Weeks, O. B. and Andrewes, A. G. In Chichester, C. O., Ed., *Advances in Chemistry of Plant Pigments*, Academic, New York 1972, Chapter 3.
7. Moiroud, A. and Gounot, A. M. *C. R. Acad. Sci. Ser. D 269* (1969) 2150.
8. Fiasson, J. L., Arpin, N., Lebreton, P. and Bouchez, M. P. *Chim. Anal (Paris) 51* (1969), 227.
9. Norgård, S. *Thesis*, Norwegian Institute of Technology, Trondheim 1972.
10. Liaaen-Jensen, S. Hertzberg, S., Weeks O. B. and Schwieter, U. *Acta Chem. Scand. 22* (1968) 1171.
11. Borch, G., Norgård, S. and Liaaen-Jensen, S. *Acta Chem. Scand. 25* (1971) 402.
12. Norgård, S., Aasen, A. J. and Liaaen-Jensen, S. *Acta Chem. Scand. 24* (1970) 2183.
13. Bowman, N. S., Rice, D. E. and Switzer, B. R. *J. Amer. Chem. Soc. 87* (1965) 4477.
14. Sievers, E. R., Ed., *Nuclear Magnetic Resonance Shift Reagents*, Academic, New York 1973.
15. a. Kjøsen, H. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 2185; b. Schwieter, U. and Liaaen-Jensen, S. *Acta Chem. Scand. 23* (1969) 1057.
16. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand. 22* (1968) 1054.
17. Francis, G. W., Norgård, S. and Liaaen-Jensen, S. *Acta Chem. Scand. B 28* (1974) 244.
18. Bartlett, L., Klyne, W., Mose, W. P., Scopes, P. M., Galasko, G. Mallams, A. K., Weedon, B. C. L., Szabolcs, J. and Toth, G. *J. Chem. Soc. C* (1969) 1527.
19. Kjøsen, H., Arpin, N. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 3053.
20. Andrewes, A. G., Liaaen-Jensen, S. and Borch, G. *Acta Chem. Scand. B 28* (1974) 737.
21. Andrewes, A. G. *Acta Chem. Scand. B 28* (1974) 137.
22. Francis, G. W. *Acta Chem. Scand. 26* (1972) 1443.
23. Canillac, N. C. *To be published.*
24. Kjøsen, H. and Liaaen-Jensen, S. *Acta Chem. Scand. 26* (1972) 4121.
25. Liaaen-Jensen, S. and Jensen, A. *Methods Enzymol 23* (1971) 586.
26. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta 210* (1970) 139.

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