for 5 min with aluminium chloride in benzene or by reaction with boron tribromide in methylene chloride for 12 h at −78°. A semi-crystalline product, 2-chloro-4-methoxybromobenzene (*7*) was obtained. NMR: 3.90 (s, OCH_3), 7−7.5 (m, aromatic H). There is no absorption corresponding to the hydrogen of the aldehyde group.

1. Forsén, S., Alm, T., Gestblom, B., Rodmar, S. and Hoffman, R. A. *J. Mol. Spectry.* **17** (1965) 13.
2. See, *e.g.*, Charton, M. *J. Am. Chem. Soc.* **91** (1969) 6649 and 624; Noyce, D. S., Bastian, N., Lau, P.T.S., Monson, R. S. and Weinstein, B. *J. Org. Chem.* **34** (1969) 1247.
3. Noelting, E. *Ber.* **37** (1904) 1015.
4. The compound gave a satisfactory C, H analysis.

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Bacterial Carotenoids XXXIV* C_{50}-Carotenoids

7.** A C_{50}-Carotenyl-D-glucoside from *Sarcina lutea*

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Several glycosides of C_{40}-carotenoids have been reported in recent years.[1,2] The occurrence of C_{50}-carotenyl glycosides has not been demonstrated before. We now report on the isolation and structure of a C_{50}-carotenyl-D-glucoside present in *Sarcina lutea*.

Details of the isolation will be reported elsewhere.[3] The glucoside (*1*) represented 20 % of the total carotenoid. *1* required as eluent from the cellulose column 30−40 % acetone in petroleum ether, $R_F = 0.45$ on Schleicher and Schüll No. 287 paper (50 % acetone in benzene), abs.max. 418, 440, and 468 nm in acetone, quantity available *ca.* 3 mg crude *1*. The peracetate (*2*), obtained on acetylation with acetic anhydride in pyridine, had $R_F = 0.63$ on the above kieselguhr paper (10 % acetone in petroleum ether) and abs. max. 417, 439, and 469 nm in acetone. The per(trideutero)acetate (*3*) was prepared in analogous manner, using hexadeuteroacetic anhydride and deuteropyridine. *2* and *3* were purified by TLC on kieselgel G (30 % acetone in petroleum ether).

The absorption spectra of *1*, *2*, and *3* were in agreement with an aliphatic nonaene chromophore. The M−92/M−106 ratio in the mass spectra of *2* and *3* was further in agreement with the values found for carotenoids with nine conjugated double bonds in the acyclic chain.[3,4] The mass spectra of the acetates (*2* and *3*) showed fragments in the lower part indicative of a hexoside. In the case of *2* these fragments corresponded to those found by Biemann *et al.*[5] for peracetylated hexoses and later encountered in acetylated carotenoid hexosides.[3,6] Analogous fragments with the appropriate mass shifts were observed for *3*, see Scheme 1. The hexose was liberated by hydrolysis of *1* (2 mg) in 0.15 N hydrogen chloride in methanol overnight, and the methyl glycoside thus obtained hydrolysed with 0.04 N polystyrene sulphonic acid.[7] The resulting reducing sugar was purified by descending paper chromatography using pyridine-ethyl acetate-water (2:5:7) [8] and spraying parallel test spots of glucose with aniline-phthalic acid reagent [9] in order to localize the sugar zone. In co-chromatography tests (System 5 [10]) with glucose and galactose ($R_{glucos} = 0.88$) the unknown hexose had $R_{glucos} = 0.99$. The paper-chromatographically purified sugar was identified as D-glucose by oxidation with D-glucose oxidase providing gluconic acid and hydrogen peroxide. The hydrogen peroxide was determined by peroxidase-catalyzed dehydrogenation of *o*-dianisidine.[11] Spectrophotometric determination and correlation with a calibration curve for D-glucose, permits quantitative determination of D-glucose on the 10 μg scale.

The molecular ion in the mass spectrum of *2* at *m/e* 1076 was in agreement with $C_{66}H_{92}O_{12}$. The molecular ion of *3* at *m/e* 1091 ($C_{66}H_{77}D_{15}O_{12}$) provided confirmation for this assignment and showed that *2* and

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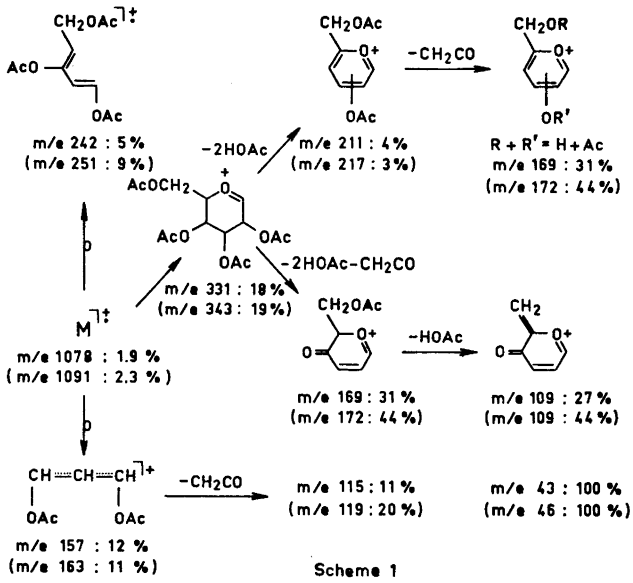
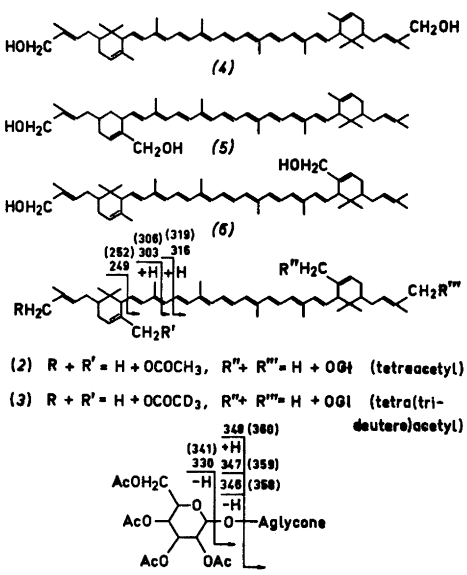


Scheme 1. Prominent ions resulting from fragmentations of the glycosidic moiety in *2* (*cf.* Ref. 5); the equivalent ions in the spectrum of *3* are given in parenthesis.

3 are pentaacetates. The identification of the molecular ions was in both cases supported by strong M−92, M−106, and M−158 ions.[4,12] Consequently the molecular formula $C_{50}H_{70}(OH)(OC_6H_{11}O_5)$ is assigned to *1*.

The identity of the glucoside (*1*) as a monoglucoside of a C_{50}-diol is further supported by its isolation from a bacterium known to produce C_{50}-carotenoids.[1,13,14] Sarcinaxanthin, a major xanthophyll of *Sarcina lutea* was for a while considered identical [13] with *dehydrogenans*-P439 (*4*),[15,16] now called decaprenoxanthin.[17] However, a re-examination [1,18] has revealed that sarcinaxanthin is an unsymmetrical isomer of *4*, either *5* or *6*, as judged from proton magnetic resonance and mass spectrometric data. The mass spectrometric fragmentation of *2* and *3* suggests that the D-glucoside discussed may be sarcinaxanthin mono-D-glucoside. Thus prominent ions in the spectrum of *2* were compatible with the fragmentations indicated in Scheme 2, assigned to in-chain cleavages of the acyclic chain near to the end group carrying the isolated acetoxy function, and in accordance with the location of the two oxygen functions at different ends of the molecule (*cf.* *6*). The possibility that one end group was hydrocarbon in nature (*cf.* *5*) and a combination of in-chain cleavage and loss of 58 (CH_2COO) mass units from the acetylated end group could be excluded on the basis of the mass shifts observed for the deuteroacetate (*3*). The *m/e* 827 ion (M−249) in *2* and the corresponding *m/e* 839 ion (M−252) in *3* were examined by high precision mass measurement, and their molecular compositions were in agreement with the theoretical values.

Other prominent ions in the spectra of *2* and *3* are indicated in Scheme 2. It is noteworthy that only ions due to cleavage of the *O*-aglycone and *O*-hexose bonds were observed in the fragmentation of the end group containing the glycosidic function, and no ions compatible with *retro*-Diels Alder fragmentation of substituted α-rings [15,16] were observed. The mass spectrometric data thus allow no preference for placing the glycosidic substituent in the 18′-position or in the extra C_5-unit (*cf.* *6*). From steric considerations the latter possibility is preferred. The implications of the present findings for the structure of sarcinaxanthin (*5* or *6*) are being further examined.[3]



Scheme 2

Scheme 2. Structures of decaprenoxanthin (*4*), sarcinaxanthin (*5* or *6*) and mass spectrometric fragmentations assigned to the acetylated D-glucoside (*2*, *3*). Bracketed values refer to *3*. Arrows point in the direction of the fragment bearing the charge.

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1. Liaaen-Jensen, S. *J. Pure Appl. Chem.* **17** (1970). *In press.*
2. Francis, G. W., Hertzberg, S., Andersen, K. and Liaaen-Jensen, S. *Phytochem.* **9** (1970) 629.
3. Arpin, N., Norgård, S., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand. To be published.*
4. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **22** (1968) 1054.
5. Biemann, K., DeJongh, D. C. and Schnoes, H. K. *J. Am. Chem. Soc.* **85** (1963) 1763.
6. Aasen, A. J., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 2605.
7. Hertzberg, S. and Liaaen-Jensen, S. *Phytochem.* **8** (1969) 1281.
8. Jensen, A. *Norw. Inst. Seaweed Research*, Report No. 9, 1965.
9. Stahl, E. *Dünnschichtschromatographie*, Springer, Berlin 1962.
10. Hertzberg, S. and Liaaen-Jensen, S. *Phytochem.* **8** (1969) 1259.
11. Richterich, R. *Klinische Chemie, Theorie und Praxis*, Basel 1965, pp. 191–194.
12. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 727.
13. Liaaen-Jensen, S., Weeks, O. B., Strang, R. H. C. and Thirkell, D. *Nature* **214** (1967) 379.
14. Thirkell, D., Strang, R. H. C. and Chapman, I. R. *J. Gen. Microbiol.* **49** (1967) 157.
15. Liaaen-Jensen, S., Hertzberg, S., Weeks, O. B. and Schwieter, U. *Acta Chem. Scand.* **22** (1968) 1171.
16. Schwieter, U. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 1057.
17. Weeks, O. B., Andrewes, A. G., Brown, B. O. and Weedon, B. C. L. *Nature* **224** (1969) 879.
18. Arpin, N., Francis, G. W. and Liaaen-Jensen, S. *Unpublished data.*

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A Low Temperature, Closed Vacuum System Distillation Technique

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Volatile compounds from tobacco and other natural products are frequently of considerable interest, but the isolation of these are usually difficult as they often occur in low concentrations mixed with large amounts of non-volatile material. The early technique of steam distillation has the disadvantage that artefacts are readily formed, while modern and milder chromatographic methods such as gel permeation chromatography are of restricted applicability due to the limited capacity of the gel. Ordinary vacuum distillation, which involves continuous pumping, often leads to substantial loss of volatile compounds,