

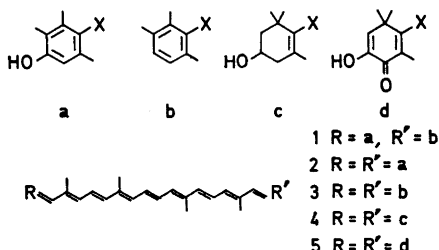
Characterization of Phenolic Carotenoids

GERD NYBRAATEN and
SYNNØVE LIAAEN-JENSEN

*Organic Chemistry Laboratories, Norwegian
Institute of Technology, University of
Trondheim, Trondheim, Norway*

After the detection of aryl carotenoids in nature¹ the existence of naturally occurring phenolic carotenoids has been predicted.² Phenolic carotenoids, namely 3-hydroxy-isorenieratene (*1*) and 3,3'-dihydroxy-isorenieratene (*2*) were first isolated two years ago by Arcamone *et al.*³ from *Streptomyces mediolani* and the structures unequivocally established by oxidative degradation as well as total synthesis.

In order to facilitate the detection of phenolic carotenoids in nature on a micro scale it was considered of interest to characterize *1* and *2* in some further detail. Samples for this purpose were kindly provided by Dr. F. Arcamone.



Spectral properties. The electronic spectra of *1* (λ_{\max} 449 and (472) nm) and *2* (λ_{\max} 451 nm) in diethyl ether exhibited less spectral fine-structure than that of isorenieratene (*3*, λ_{\max} (430), 451 and 487 nm). In methanol still more rounded spectra were observed for the phenols *1* (λ_{\max} 450, (474) nm) and *2* (λ_{\max} 453 nm). On addition of traces of alkali a certain bathochromic effect and further loss of fine-structure was observed for the spectrum of the diol (*2*).

IR spectra (KBr) of *1* and *2* had characteristic absorption ascribed to the phenolic hydroxy groups at 3400 (broad, OH stretching) and 1250 and 1080 (OH deformation, C—O stretching) cm^{-1} , Fig. 1.

Mass spectra of *1* and *2* showed the following characteristic fragments: M, M—92, M—106, and M—158; no M—18 peak. As for the aryl carotenes,⁴ characteristic cleavage of the 7-double bond with hydrogen transfer to the charged fragment caused strong m/e 149 (100%) ions in the spectra of both *1* and *2*. The M—92/M—106 ratio for *1* was 3.5 and for *2* 1.8, that is in the expected⁴ range.

Other physical properties. In co-chromatography tests on circular kieselguhr paper⁶ (all R_F -values refer to this paper) *2* with $R_F=0.69$ in 20% AP (AP=acetone in petroleum ether) and $R_F=0.27$ in 10% AP was more strongly adsorbed than zeaxanthin (*4*, $R_F=0.92$ in 20% AP) and also more strongly adsorbed than astacene (*5*, $R_F=0.44$ in 10% AP). For comparison *1* had $R_F=0.84$ in 10% AP. A characteristic feature of the diphenol *2*, not observed for the monophenol *1*, was the development of a bluish colour on air-drying of the chromatographic paper; cf. oxidation studies below. Alternatively spraying with diazotized benzidine reagent⁷ of the freshly developed paper chromatogram was performed. The zones of the phenols *1* and *2* obtained blue-brown and strong blue colours respectively, the non-enolic zeaxanthin (*4*) became invisible and the enolic astacene (*5*) attained a greyish colour. The above colour reactions are therefore useful in indicating the presence of phenolic carotenoids.

In partition⁸ between petroleum ether and 85% aqueous methanol *1* gave the following ratios 96:4 (neutral) and 82:18 in the presence of traces of alkali. The corresponding values for *2* could not be determined due to solubility problems. In alkaline aqueous methanol the spectrum of *1* in each of the two phases remained unchanged, spectra of *2* were markedly influenced by the base.

Chemical reactions. Since saponification is usually involved in isolation of carotenoids it was of interest to check the stability of *1* and *2* during such standard⁹ treatment. The results indicated that *1* was more stable than *2*, but satisfactory recoveries were in no case obtained. In the work-up procedure acidification of the hypophase to pH 4 was required to permit complete transfer of *2* to ether. It is recommended that the saponification step be avoided in the isolation of phenolic carotenoids.

The acetylation reaction proceeded smoothly. Thus *1* gave the monoacetate

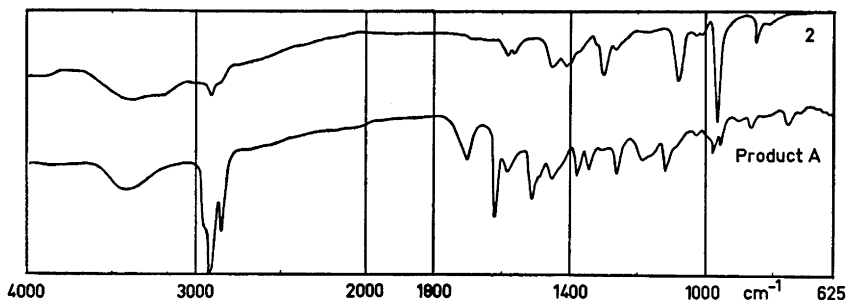


Fig. 1. IR spectra (KBr) of 3,3'-dihydroxy-isorenieratene (2) and of Product A.

($R_F=0.88$ in 5% AP) in quantitative yield.

Methylation with diazomethane¹⁰ of 1 and 2 could surprisingly enough not be effected; cf. lack of methylation of astacene (5).⁹

Treatment of 1 and 2 with *p*-chloranil¹¹ gave no defined products. Moreover, 1 gave no oxidation products with silver carbonate-celite,¹² whereas 2 gave a dark blue oxidation product (Product A), formed in quantitative yield according to the following procedure: 2 (0.2 mg) in dry acid- and ethanol-free chloroform (3 ml) was refluxed with Ag_2CO_3 -celite (10 mg) for 2 h with mechanical stirring, then at room temperature for 15 h. Inorganic material was removed by filtration: pigment recovery ca. 50%. Product A, $R_F=0.29$ in 20% AP (colour unstable), λ_{max} 562 nm in benzene, 580 nm in chloroform (Fig. 2) or pyridine, exhibited absorption at longer wavelength than any known carotenoid and was very unstable.

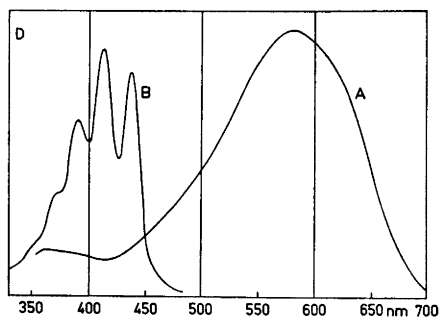
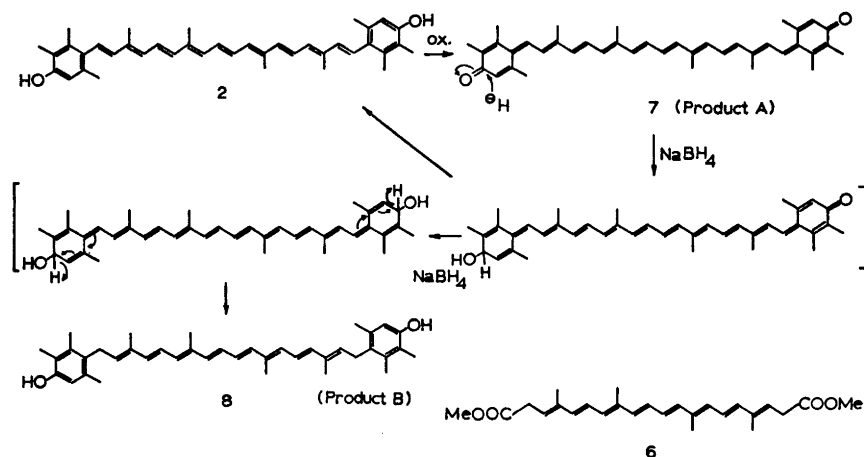


Fig. 2. Visible light absorption spectra in chloroform of Product A and Product B.

Repeated attempts to obtain its mass spectrum failed. The IR spectrum (KBr, Fig. 1) had characteristic absorption at 1625 cm^{-1} , in support of a quinonoid structure.¹³ Product A was unstable under conditions required to form an oxime¹⁴ or a dinitrophenylhydrazone¹⁴ and towards acetylation conditions. On treatment of Product A with sodium borohydride in ethanol two new products were quickly formed, one orange pigment identified as 2 (co-chromatography test, electronic spectrum and colour reactions) and a major yellow product (Product B) with $R_F=0.85$ in 20% AP: λ_{max} 362, 381, 402 and 427 nm in petroleum ether and 372, 391, 412 and 438 nm in chloroform (Fig. 2); m/e 562 (M), M-92, M-106, m/e 149. The electronic spectrum of Product B was directly compared with that of dihydromethylbixin (6). 6 was prepared for comparison by a Wittig reaction of crocetinindial and carbomethyloxymethylenetriphenylphosphorane to give all-*trans* methyl bixin, followed by Zn-acetic acid reduction¹⁵ to 6. The spectrum in visible light of Product B was conform with that of 6 (chloroform); the maxima of 6 being displaced 3 nm towards shorter wavelengths. Product B gave an acetate ($R_F=0.76$ in 5% AP) on acetylation. Structure 7 is considered for Product A and structure 8 for Product B, see Scheme 1. Characterization of Products A and B on a larger scale is desirable.

In conclusion phenolic carotenoids appear to exhibit characteristic physical and chemical properties which should facilitate their future identification.

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Scheme 1.

Milano, for samples of 1 and 2 and to him and his co-workers for letting us undertake the present investigation.

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14. Aasen, A. J. and Liaaen Jensen, S. *Acta Chem. Scand.* **21** (1967) 2185.
15. Kuhn, R. and Brockmann, H. *Ber.* **66** (1933) 828.

Received December 23, 1970.

1. Yamaguchi, M. *Bull. Chem. Soc. Japan.* **30** (1957) 111.
2. Liaaen Jensen, S. *Pure Applied Chem.* **14** (1967) 227.
3. Arcamone, F., Camerino, B., Cotta, E., Franceschi, G., Grein, A., Penco, S. and Spalla, C. *Experientia* **24** (1968) 241.
4. Kjösen, H., Liaaen-Jensen, S. and Enzell, C. R. *Acta Chem. Scand. In press.*
5. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **22** (1968) 1054.
6. Jensen, A. and Liaaen Jensen, S. *Acta Chem. Scand.* **13** (1959) 1863.
7. Stahl, E. *Dünnschicht-Chromatographie, Ein Laboratoriumshandbuch*, Springer, Berlin 1962, p. 322.
8. Petracek, F. J. and Zechmeister, L. *Anal. Chem.* **28** (1956) 1484.
9. Aasen, A. J. and Liaaen Jensen, S. *Acta Chem. Scand.* **20** (1966) 1970.
10. de Boer, T. J. and Backer, H. J. *Rec. Trav. Chim.* **73** (1954) 229.
11. Liaaen Jensen, S. *Acta Chem. Scand.* **19** (1965) 1166.
12. Fetizon, M. and Golfier, M. *Compt. Rend.* **267** (1968) 900.
13. Bellamy, L. J. *The Infra-red Spectra of Complex Molecules*, Methuen, London 1964.

Derivatives of Thio- and Selenocarbonic Acids

I. An Adduct of Trithiocarbonate Ion and Carbon Disulfide

LARS HENRIKSEN

Chemical Laboratory II (General and Organic Chemistry), University of Copenhagen, The H. C. Ørsted Institute, DK-2100 Copenhagen, Denmark

The previously reported¹ formation of dialkyl triselenocarbonates from carbon diselenide, an alkyl halide and an oxygen base requires that an oxygen-selenium exchange takes place at the carbonate group. The restriction of this reaction to dimethyl sulfoxide (DMSO) and related

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