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METHOXYSPHEROIDENE AND METHOXYSPHEROIDENONE, TWO CAROTENOIDS FROM *RHODOPSEUDOMONAS SPHEROIDES*

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Key Word Index—*Rhodopseudomonas spheroides*; Rhodospirillaceae; 1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-carotene; 1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one.

Abstract—Two new carotenoids isolated from *Rhodopseudomonas spheroides* (Rhodospirillaceae) have been identified as methoxyspheroidene (1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-carotene) obtained from anaerobic cultures and methoxyspheroidenone (1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one) recovered from aerobic cultures.

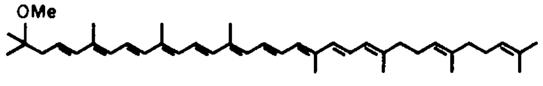
INTRODUCTION

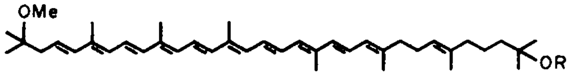
Purple non-sulphur photosynthetic bacteria of the Rhodospirillaceae are characterized by the presence of acyclic carotenoids with tertiary methoxy and hydroxy substituents at C-1 and C-1′. [1] Thus in *Rhodopseudomonas spheroides* grown under anaerobic conditions, the main pigments are spheroidene ("Pigment Y", 1-methoxy-3,4-didehydro-1,2,7′,8′-tetrahydro-ψ,ψ-carotene, **1**) and hydroxyspheroidene ("Hydroxy-Y", 1′-methoxy-3′,4′-didehydro-1,2,7,8,1′,2′-hexahydro-ψ,ψ-caroten-1-ol, **2**) [2,3], and the presence of "methoxyspheroidene" (1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-carotene, **3**) has been commented upon [4,5]. Details of the characterization of this compound are now presented.

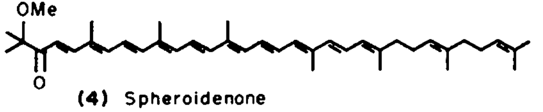
It is well known that in *R. spheroides* in the presence of oxygen, spheroidene and hydroxyspheroidene are converted into their 2-oxo derivatives, spheroidenone ("Pigment R", 1-methoxy-3,4-didehydro-1,2,7′,8′-tetrahydro-ψ,ψ-caroten-2-one, **4**) and hydroxyspheroidenone ("Hydroxy-R", 1′-hydroxy-1-methoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one, **5**) [2,6–8]. Similar formation of the 2-oxo derivative of methoxyspheroidene, has now been observed, and the product, "methoxyspheroidenone" (1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one, **6**) has been characterized by its chromatographic properties and absorption spectrum, and by the MS of the compound and its derivatives.

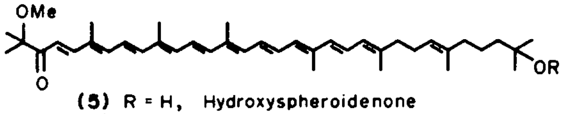
RESULTS AND DISCUSSION

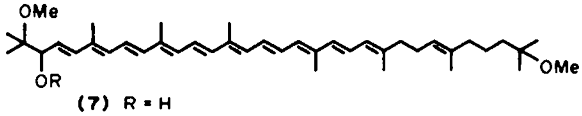
Methoxyspheroidene. This compound, isolated from anaerobic cultures of *R. spheroides*, had an absorption spectrum identical to those of spheroidene and hydroxyspheroidene. On silica gel it was adsorbed more strongly than spheroidene, but much less strongly than hydroxyspheroidene; its chromatographic polarity was similar to that of spirilloxanthin (1,1′-dimethoxy-3,4,3′,4′-tetradehydro-1,2,1′,2′-tetrahydro-ψ,ψ-carotene), suggesting that it might be a dimethoxy compound. This was confirmed by the MS, which showed the molecular weight to be 600 ($C_{42}H_{64}O_2$), and contained major fragment ions at *m/e* 568 and 536 due to losses of one and two molecules of methanol, respectively. The base peak of the spectrum at *m/e* 73 and a fragment ion at *m/e* 527 due to loss of 73 m.u. confirmed the presence of the 1-methoxy-1,2-dihydro-3,4-didehydro end group. "Bis-allylic" cleavage of the C-7′,8′ bond gave the expected M-169 fragment at *m/e* 431. Multiple losses of these fragments with toluene (92 m.u.) and especially xylene (106 m.u.) were also observed.


(1) Spheroidene


(2) R = H, Hydroxyspheroidene
(3) R = Me, Methoxyspheroidene


(4) Spheroidenone


(5) R = H, Hydroxyspheroidenone
(6) R = Me, Methoxyspheroidenone


(7) R = H
(8) R = Ac

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The properties of this compound were fully in accord with the structure **3**, 1,1′-dimethoxy-3,4-didehydro-1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-carotene. It has previously been detected in cultures of *Rhodospirillum rubrum* grown in the presence of diphenylamine [4], but this is the first detailed report of its occurrence in a photosynthetic bacterium cultured under normal conditions, i.e. with no inhibitor present. Only small amounts of methoxyspheroidene were present, usually about 1% of the amount of spheroidene.

Methoxyspheroidenone. When air was admitted to the previously anaerobic cultures of *R. spheroides*, substantial conversion of the yellow spheroidene and hydroxyspheroidene into their somewhat more polar red derivatives spheroidenone and hydroxyspheroidenone occurred after 24 hours. Similar conversion of methoxyspheroidene into a more polar red compound was also observed. This red product had an absorption spectrum identical to those of spheroidenone and hydroxyspheroidenone, with λ_{max} (ethanol) at approx. 487 nm, shifting to 428, 453 and 484 nm after treatment with $NaBH_4$. This suggested that this compound was the 2-oxo derivative of methoxyspheroidene, i.e. methoxyspheroidenone, and this was confirmed by MS. The parent ion, M^+ was at *m/e* 614 ($C_{42}H_{62}O_3$), with major fragment ions at *m/e* 582 (M-MeOH), 550 (M-2 × MeOH), 541 (M-73) and 445 (M-169). Multiple losses of these fragments with toluene and xylene were also observed.

The MS of the $NaBH_4$ reduction product **7** showed the molecular weight to be 616 ($C_{42}H_{64}O_3$), indicating the reduction of one carbonyl group to a hydroxyl group. This was confirmed by the presence of a fragment ion at *m/e* 598 due to loss of water. The hydroxyl group could be acetylated to give a product **8** with molecular weight 658 ($C_{44}H_{66}O_4$). All the available data were therefore consistent with structure **6**, 1,1′-dimethoxy-3,4-didehydro1,2,1′,2′,7′,8′-hexahydro-ψ,ψ-caroten-2-one, for the natural oxocarotenoid. The natural occurrence of this compound has not been reported previously.

The ratio of methoxyspheroidenone to methoxyspheroidene was similar to the ratios of spheroidenone to spheroidene and hydroxyspheroidenone to hydroxyspheroidene. It thus appears that the enzyme responsible for introducing the oxo group is able to deal equally well with spheroidene and its 1′-hydroxy-1′,2′-dihydro and 1′-methoxy-1′,2′-dihydro derivatives.

EXPERIMENTAL

Organism and culture conditions. Cultures of *Rhodopseudomonas spheroides* (N.C.I.B. 8253), obtained from the National Collection of Industrial Bacteria, Aberdeen, were grown in the light as previously described [9]. Methoxyspheroidene was obtained from cultures grown anaerobically for 6 days, methoxyspheroidenone from cultures grown anaerobically for 3–4 days and then exposed to air (no shaking) for 24 hr.

Pigment extraction and purification. Cells were harvested by centrifugation and the pigments extracted with Me_2CO and MeOH and purified by standard procedures [5]. The carotenoids described were both eluted from a column of neutral alumina (Woelm, Brockmann Grade III) with 20% Et_2O in petrol (bp 40–60°). Purification by successive TLC on Si gel G (10% Et_2O in petrol), MgO-Kieselguhr G (1:1; Me_2CO–C_6H_6-petrol, 1:1:5) and Si gel G (15% Et_2O in petrol) gave methoxyspheroidene (**3**) R_f 0.35, 0.60, 0.60 respectively; λ_{max}^{EtOH} nm: 428, 453 and 485; MS: M^+ at *m/e* 600 (18%, $C_{42}H_{64}O_2$), fragment ions at *m/e* 568 (8%, M-MeOH, m^* 538; $568^2/600 = 537.7$), 536 (7%, M-2 × MeOH, m^* 506; $536^2/568 = 505.8$), 527 (2%, M-73, m^* 463, $527^2/600 = 462.8$), 508 (3%, M-toluene, m^* 430; $508^2/600 = 430.1$), 494 (16%, M-xylene), 462 (6%, M-MeOH–xylene), 431 (5%, M-169, m^* 310; $431^2/600 = 309.6$), 430 (1,5%, M-2 × MeOH–xylene), 421 (2%, M-xylene-73, m^* 361; $421^2/494 = 361.3$) and 73 (100%) and methoxyspheroidenone (**6**) R_f 0.20, 0.50, 0.40 respectively; λ_{max}^{EtOH} approx. 487 nm; MS: M^+ at *m/e* 614 (20%, $C_{42}H_{62}O_3$), fragment ions at *m/e* 582 (3%, M-MeOH, m^* 552; $582^2/614 = 551.7$), 550 (1%, M-2 × MeOH, m^* 520; $550^2/582 = 519.8$), 541 (1%, M-73, m^* 477; $541^2/614 = 476.7$), 522 (3%, M-toluene), 508 (20%, M-xylene), 490 (1%, M-toluene–MeOH), 476 (2%, M-xylene–MeOH), 445 (7%, M-169, m^* 322.5; $445^2/614 = 322.5$) 435 (2%, M-xylene-73, m^* 372.5; $435^2/508 = 372.5$), 413 (1%, M-MeOH-169), 339 (10%, M-xylene-169, m^* 227; $339^2/508 = 226.8$) and 73 (100%). The $NaBH_4$ reduction product **7** was purified by TLC on Si gel G (Et_2O–petrol, 1:1) R_f 0.45; λ_{max}^{EtOH} nm: 428, 453 and 484; MS: M^+ at *m/e* 616 (24%, $C_{42}H_{64}O_3$) and fragment ions at *m/e* 598 (3%, M-H_2O, m^* 581; $598^2/616 = 580.5$), 584 (4%, M-MeOH, m^* 554; $584^2/616 = 553.7$), 566 (5%, M-H_2O–MeOH, m^* 549 and 536; $566^2/584 = 548.6$, $566^2/598 = 535.7$), 543 (1%, M-73, m^* 479; $543^2/616 = 478.7$), 510 (20%, M-xylene), 492 (4%, M-xylene–H_2O, m^* 475; $492^2/510 = 474.6$), 447 (3%, M-169, m^* 324; $447^2/616 = 324.4$), 437 (8%, M-xylene-73, m^* 374; $437^2/510 = 374.4$), 419 (3%, M-xylene-H_2O-73), 405 (6%, M-xylene–MeOH-73), 341 (6%, M-xylene-169, m^* 228; $341^2/510 = 228.0$), 73 (100%). Its acetate **8** (Ac_2O/C_5H_5N) was purified by TLC on Si gel G with 10% Et_2O in petrol R_f 0.30; λ_{max}^{EtOH} nm: 427, 453 and 484; MS: M^+ at *m/e* 658 (10%, $C_{44}H_{66}O_4$), fragment ion at *m/e* 598 (4%, M-AcOH, m^* 544; $598^2/658 = 543.5$).

Spectra. Absorption and mass spectra were obtained as previously described [10]. Mass spectra were determined by Mr. G. Harriman.

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