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# THE MONO- AND DIMETHOXY-CAROTENOIDS OF DIPHENYLAMINE-INHIBITED CULTURES OF *RHODOSPIRILLUM RUBRUM*

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**Abstract**—Eight monomethoxy- and six dimethoxy-carotenoids have been isolated from diphenylamine-inhibited cultures of *Rhodospirillum rubrum.* Of these, 3,4,11′,12′-tetrahydrospheroidene (III), 11′,12′-dihydrospheroidene (IV), 3,4-dihydrospheroidene (V), spheroidene (VI), 3,4-dihydroanhydrorhodovibrin (VII), anhydrorhodovibrin (VIII), 3′,4′,7′,8′-tetrahydrospirilloxanthin (XI), 3,4,3′,4′-tetrahydrospirilloxanthin (XII), 3,4-dihydrospirilloxanthin (XIII) and spirilloxanthin (XIV) have been characterized by their electronic absorption and mass spectra, and chromatographic properties. The other compounds present have been tentatively identified as 1-methoxy-1,2-dihydrophytoene (I), 1-methoxy-1,2-dihydrophytofluene (II), 3,4,3′,4′,7′,8′,11′,12′-octahydrospirilloxanthin (IX), and 3,4,3′,4′,7′,8′-hexahydrospirilloxanthin (X).

## INTRODUCTION

*Rhodospirillum rubrum*, a purple photosynthetic bacterium of the Athiorhodaceae, when grown anaerobically in the light accumulates large quantities of the dimethoxy-carotenoid spirilloxanthin (XIV).[1–3] In the presence of diphenylamine (DPA), however, spirilloxanthin synthesis is largely inhibited, and a range of more saturated carotenoids, especially phytoene, accumulates.[4] Several of these pigments have been identified and suggested as possible intermediates in the biosynthesis of spirilloxanthin. A series of kinetic studies enabled Jensen *et al.*[5,6] to postulate a possible pathway of spirilloxanthin biosynthesis in *R. rubrum.*

A re-examination, by modern techniques, of the pigments of DPA-inhibited cultures of *R. rubrum* has shown that, in addition to the hydrocarbons phytoene, phytofluene, 7,8,11,12-tetrahydrolycopene and neurosporene,[7,8] a wide range of monohydroxy-, monomethoxy-, hydroxymethoxy- and dimethoxy-carotenoids also occur. The structures of some of these compounds have been reported[7–12] and alternative schemes proposed for spirilloxanthin biosynthesis.[9] We now report the results of a survey of the mono- and dimethoxy-carotenoids of DPA-inhibited cultures of *R. rubrum.*

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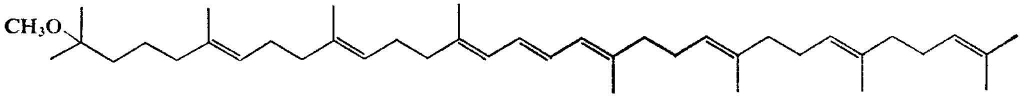
[5] S. L. Jensen, G. Cohen-Bazire, T. O. M. Nakayama and R. Y. Stanier, *Biochim. Biophys. Acta* **29**, 477 (1958).
[6] S. L. Jensen, G. Cohen-Bazire and R. Y. Stanier, *Nature* **192**, 1168 (1961).
[7] B. H. Davies, E. A. Holmes, D. E. Loeber, T. P. Toube and B. C. L. Weedon, *J. Chem. Soc.* (c) 1266 (1969).
[8] B. H. Davies, *Biochem. J.* **116**, 93 (1970).
[9] B. H. Davies, *Biochem. J.* **116**, 101 (1970).
[10] H. C. Malhotra, G. Britton and T. W. Goodwin, *Phytochem.* **8**, 1047 (1969).
[11] H. C. Malhotra, G. Britton and T. W. Goodwin, *FEBS Letters* **6**, 334 (1970).
[12] S. L. Jensen and A. Jensen, *Progr. Chem. Fats Lipids* **8**, 165 (1965).

## RESULTS

Two series of carotenoids were obtained from cultures of *Rhodospirillum rubrum* grown anaerobically in the light in the presence of diphenylamine. These series had chromatographic properties of monomethoxy- and dimethoxy-carotenoids, respectively.

### *Monomethoxy-carotenoids*

The electronic spectra of the carotenoids of the monomethoxy fraction showed the presence of compounds with chromophores of 3, 5, 7, 8, 9, 10, 11, and 12 conjugated double bonds. The structures of those compounds isolated in sufficient quantities were further examined by mass spectrometry. The other members of the series, present only in minute amounts, were assigned tentative structures from their chromatographic behaviour and electronic spectra.



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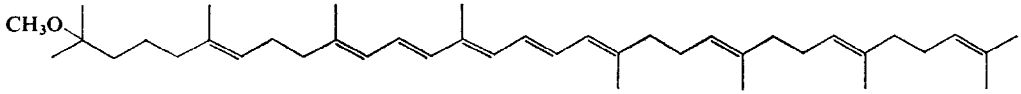

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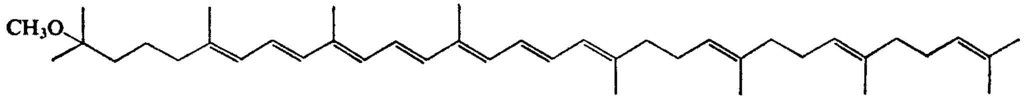

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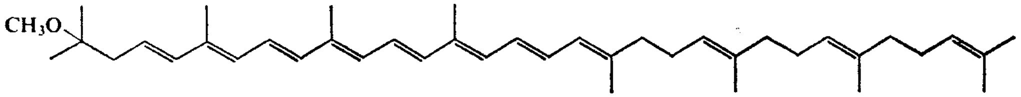

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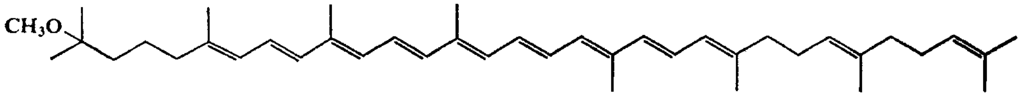

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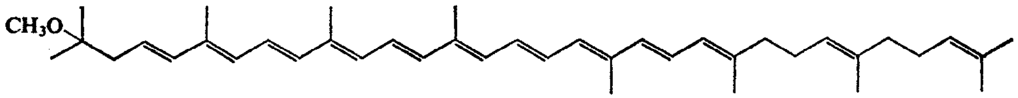

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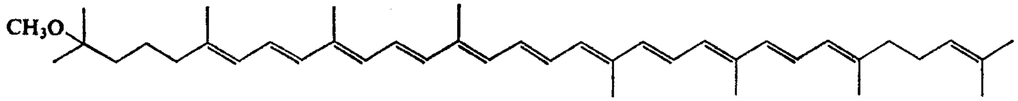


(VII)

(i) "*Methoxyphytoene*" (*I*)

This compound, which was isolated in very minute amounts, had an u.v. spectrum characteristic of a phytoene derivative, with $\lambda_{max}$ at 275, 285, 296 nm. From this, and its chromatographic properties, this compound is tentatively identified as a methoxy derivative of phytoene, probably, on biogenetic grounds, 1-methoxy-1,2-dihydrophytoene (I).

(ii) "*Methoxyphytofluene*" (*II*)

Closely associated with the above compound (I) was another minor compound identified by its u.v. spectrum ($\lambda_{max}$ 331, 348, 367 nm) and characteristic strong greenish fluorescence in u.v. light as a phytofluene derivative. This compound was also not isolated in sufficient quantities for mass spectrometric analysis, but is tentatively identified as a methoxy derivative of phytofluene, on biogenetic grounds probably 1-methoxy-1,2-dihydrophytofluene (II).

(iii) 3,4,11′,12′-*Tetrahydrospheroidene* (*III*)

Larger quantities were isolated of a compound which had chromatographic properties similar to those of I and II, and had an electronic spectrum ($\lambda_{max}$ 374, 394, 418 nm) characteristic of an unsymmetrical conjugated heptaene chromophore.[7] The mass spectrum had the parent ion, $M^+$ at *m/e* 572 ($C_{41}H_{64}O$) and fragmentation pattern identical to that described by Davies *et al.*[7] for 3,4,11′,12′-tetrahydrospheroidene (III).

(iv) 11′,12′-*Dihydrospheroidene* (*IV*)

A carotenoid with the chromatographic properties of a monomethoxy compound and electronic spectrum ($\lambda_{max}$ 392, 413, 439 nm) indicative of the unusual conjugated octaene chromophore was also isolated. The mass spectrum showed the molecular ion at *m/e* 570 ($C_{41}H_{62}O$), and fragmentation pattern identical to that described by Davies *et al.*[7] for 11′,12′-dihydrospheroidene (IV).

(v) 3,4-*Dihydrospheroidene* (1-*Methoxy*-1,2-*dihydroneurosporene*) (*V*)

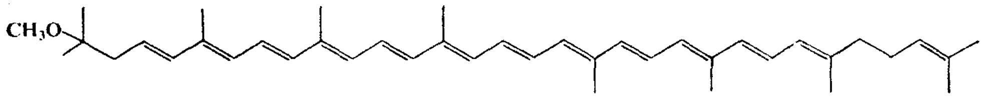
A compound was isolated which had chromatographic properties and visible spectrum ($\lambda_{max}$ 414, 438, 468 nm) characteristic of a methoxy derivative of neurosporene. The mass spectrum showed the molecular ion $M^+$ at *m/e* 570 ($C_{41}H_{62}O$) and a major fragment ion at *m/e* 538 (metastable at *m/e* 508; $538^2/570 = 507{\cdot}8$), due to loss of methanol. A strong fragment ion was observed at *m/e* 433 (M-137, metastable at *m/e* 329; $433^2/570 = 328{\cdot}9$) due to cleavage of the "bis-allylic" C-7′,8′ bond. Losses of toluene (92 mass units) and xylene (106 mass units) from the parent ion were also observed. The loss of 88 mass units from the *m/e* 433 ion (fragment ion at *m/e* 345, metastable at *m/e* 275; $345^2/433 = 274{\cdot}9$) is similar to that discussed by Davies *et al.*[7] in the mass spectrum of 3,4,11′,12′-tetrahydrospheroidene. This, together with the absence of any major fragments involving the loss of 73 mass units, indicates the presence of the 1-methoxy-1,2,3,4-tetrahydro end group, thus confirming the structure of this new compound as 3,4-dihydrospheroidene (1-methoxy-1,2-dihydroneurosporene) (V).

(vi) *Spheroidene* (*VI*)

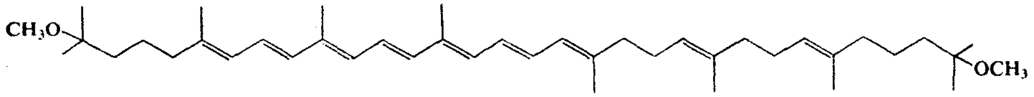
The isolation and characterization of spheroidene from DPA-inhibited cultures of *R. rubrum* has previously been reported.[7,10]

(vii) 1-*Methoxy*-1,2-*dihydrolycopene* (3,4-*Dihydroanhydrorhodovibrin*) (*VII*)

Small quantities were isolated of a compound closely associated with spheroidene, but with $\lambda_{max}$ at 444, 470, 501 nm characteristic of a lycopene derivative. The mass spectrum was too weak for full interpretation, but had the molecular ion $M^+$ at *m/e* 568 ($C_{41}H_{60}O$), and weak fragment ions were observed at *m/e* 536 (M-$CH_3OH$), 480 (M-88) and 476 (M-92). No other major fragment ions, and no metastable ions were detected. This compound is tentatively identified as 1-methoxy-1,2-dihydrolycopene (3,4-dihydroanhydrorhodovibrin) (VII).



(VIII)



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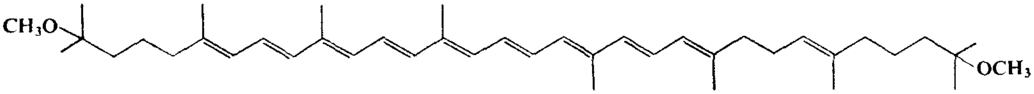

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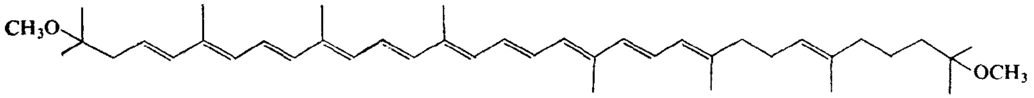

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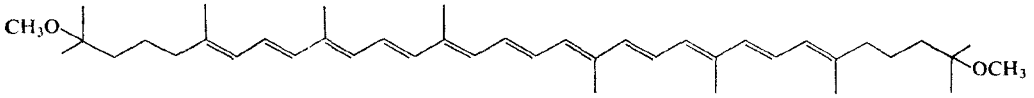

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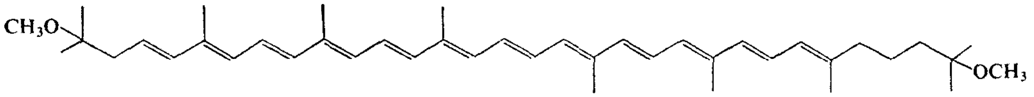
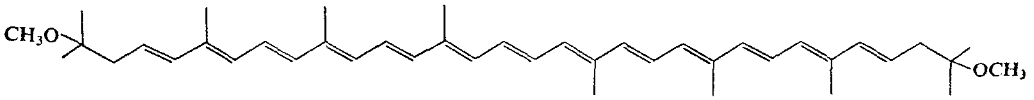


(XIII)



(XIV)

(viii) *Anhydrorhodovibrin (VIII)*

A compound was obtained which had chromatographic properties, visible spectrum ($\lambda_{max}$ 453, 480, 512 nm) and mass spectrum identical to those of anhydrorhodovibrin from normal cultures of *R. rubrum*.

### *Dimethoxy-carotenoids*

The electronic spectra of the carotenoids of the dimethoxy series showed the presence of compounds with 7, 9, 10, 11, 12 and 13 conjugated double-bond chromophores. Carotenoids with 3, 5 or 8 conjugated double-bond chromophores were not detected. The structures of the compounds present were investigated, when possible, by mass spectrometry.

(i) 3,4,3′,4′,7′,8′,11′,12′-*Octahydrospirilloxanthin* (*IX*)

A compound, isolated in quantities too small for mass spectrometric analysis, had the chromatographic properties of a dimethoxy-carotenoid, and an electronic spectrum ($\lambda_{max}$ 375, 395, 420 nm) characteristic of an unsymmetrical conjugated heptaene chromophore. This compound was tentatively identified as 3,4,3′,4′,7′,8′,11′,12′-octahydrospirilloxanthin (IX).

(ii) 3,4,3′,4′,7′,8′-*Hexahydrospirilloxanthin* (1,1′-*Dimethoxy*-1,1′,2,2′-*tetrahydroneurosporene* (*X*)

A dimethoxy-carotenoid with electronic spectrum ($\lambda_{max}$ 414, 438, 467 nm) characteristic of a neurosporene derivative (conjugated nonaene chromophore) was also obtained. From the very small amount of material available, only a weak mass spectrum was obtained which showed the molecular ion at *m/e* 602 ($C_{42}H_{66}O_2$) in agreement with the tentative identification of the compound as 3,4,3′,4′,7′,8′-hexahydrospirilloxanthin (X). The mass spectrum was too weak for full interpretation.

(iii) 3′,4′,7′,8′-*Tetrahydrospirilloxanthin* (1′-*Methoxy*-1′,2′-*dihydrospheroidene*) (*XI*)

This carotenoid had an electronic spectrum ($\lambda_{max}$ 425, 453, 483 nm) identical to those of spheroidene and hydroxyspheroidene, but had the chromatographic properties of a dimethoxy compound. The mass spectrum had a molecular ion $M^+$ at *m/e* 600 ($C_{42}H_{64}O_2$). Losses of 1 and 2 mol of methanol respectively gave rise to major fragment ions at *m/e* 568 (metastable at *m/e* 538; $568^2/600 = 537{\cdot}7$) and 536. A strong loss of 73 mass units from the parent ion (fragment ion at *m/e* 527, metastable at *m/e* 463; $527^2/600 = 462{\cdot}9$) was indicative of the presence of a 1-methoxy-1,2-dihydro- end group adjacent to a C-3,4 double bond. The structure of the other end of the molecule was indicated by the presence of major fragment ions at *m/e* 512 (M-88) and 431, due to loss of 169 mass units from the molecular ion by cleavage of the "bis-allylic" C-7′,8′ bond (metastable at *m/e* 310; $431^2/600 = 309{\cdot}6$). Fragment ions produced by losses of 32, 73, 88 or 169 mass units and toluene (92 mass units) or xylene (106 mass units) were also observed. The mass spectral and other data are consistent with the formulation of this new compound as 3′,4′,7′,8′-tetrahydrospirilloxanthin (1′-methoxy-1′,2′-dihydrospheroidene) (XI). This compound has also been isolated from *Rhodopseudomonas spheroides*.[13]

(iv) 3,4,3′,4′-*Tetrahydrospirilloxanthin* (1,1′-*Dimethoxy*-1,2,1′,2′-*tetrahydrolycopene*) (*XII*)

Closely associated with 3′,4′,7′,8′-tetrahydrospirilloxanthin (XI) was a pigment with electronic spectrum ($\lambda_{max}$ 444, 470, 501 nm) characteristic of a lycopene derivative. The mass spectrum showed the molecular ion at *m/e* 600 ($C_{42}H_{64}O_2$) and fragmentation pattern identical with that given by Enzell, Francis and Jensen[14] for 3,4,3′,4′-tetrahydrospirilloxanthin (XII).

[13] H. C. MALHOTRA, G. BRITTON and T. W. GOODWIN, unpublished results, 1968.

[14] C. R. ENZELL, G. W. FRANCIS and S. L. JENSEN, *Acta Chem. Scand.* **23**, 727 (1969).

### (v) 3,4-*Dihydrospirilloxanthin* (*XIII*)

A compound was obtained with electronic spectrum ($\lambda_{max}$ 453, 480, 512 nm) indicative of the presence of a conjugated 12-double bond chromophore similar to that of anhydrorhodovibrin (VIII). The mass spectrum had the molecular ion ($M^+$) at *m/e* 598 ($C_{42}H_{62}O_2$) and major fragment ions at *m/e* 566 (metastable at *m/e* 536; $566^2/598 = 535{\cdot}7$) and 534 due to respective losses of one and two molecules of methanol. Loss of 73 mass units (fragment ion at *m/e* 525, metastable at *m/e* 461; $525^2/598 = 460{\cdot}9$) showed the presence of the 1′-methoxy-1′,2′-dihydro-3′,4′-dehydro- end group, and a loss of 88 mass units (fragment ion at *m/e* 510) was also observed, and was possibly due to cleavage of the C-3,4 bond at the other end of the molecule. Multiple losses of 32, 73, or 88 mass units, together with toluene (92) or xylene (106) were also observed. The mass spectral data are consistent with structure XIII, 3,4-dihydrospirilloxanthin.

### (vi) *Spirilloxanthin* (*XIV*)

The final compound in this series was shown by its chromatographic properties, visible spectrum ($\lambda_{max}$ 464, 490, 523 nm) and mass spectrum to be identical to spirilloxanthin (XIV) obtained from cultures of *Rhodospirillum rubrum* grown under normal conditions.

## DISCUSSION

Under conditions of DPA inhibition, *Rhodospirillum rubrum* is thus capable of forming eight monomethoxy-carotenoids with chromophores of 3, 5, 7, 8, 9, 10, 11 and 12 conjugated double bonds respectively. The presence of some of these compounds has been reported[7,9,10] (3,4,11′,12′-tetrahydrospheroidene (III), 11′,12′-dihydrospheroidene (IV), spheroidene (VI) and anhydrorhodovibrin (VIII)), and a recent paper by Davies[9] has confirmed the presence of 3,4-dihydrospheroidene (V) and 3,4-dihydroanhydrorhodovibrin (VII). The monomethoxy derivatives of phytoene and phytofluene (I, II) have not previously been reported.

Of the dimethoxy series, only spirilloxanthin (XIV) has previously been shown to be present in *R. rubrum*. This is the first report of the natural occurrence of 3,4,3′,4′,7′,8′,11′,12′-octahydrospirilloxanthin (IX), 3,4,3′,4′,7′,8′-hexahydrospirilloxanthin (X), 3′,4′,7′,8′-tetrahydrospirilloxanthin (XI) and 3,4-dihydrospirilloxanthin (XIII). 3,4,3′,4′-Tetrahydrospirilloxanthin (XII) has been shown to be the major carotenoid of a photolithotropic bacterium (RG3),[15] but has not previously been detected in *R. rubrum*. Possible biosynthetic implications of the presence of these and other compounds in DPA-inhibited cultures of *R. rubrum* will be considered elsewhere.

## EXPERIMENTAL

### *Cultures and Cultural Conditions*

Cultures and cultural conditions were as previously described.[10]

### *Pigment Extraction and Purification*

Cells were harvested, pigments extracted and the monomethoxy and dimethoxy fractions obtained as previously described.[10] The monomethoxy and dimethoxy compounds were separated by TLC on MgO–Kieselgur G (1:1) with 10% and 15% respectively of acetone in light petroleum as developing solvent. The individual compounds were further purified by successive TLC on MgO–Kieselgur G (1:1) and Silica gel G with the solvent systems shown in Table 1.

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[15] A. J. AASEN and S. L. JENSEN, *Acta Chem. Scand.* **21**, 371 (1967).

TABLE 1. SOLVENT SYSTEMS USED FOR TLC PURIFICATION OF MONOMETHOXY- AND DIMETHOXY-CAROTENOIDS OF DPA-INHIBITED *Rhodospirillum rubrum*

| Compound | Third TLC (MgO–Kieselgur G, 1:1) | Fourth TLC (Silica Gel G) |
|---|---|---|
| "Methoxyphytoene" (I) | 8% B/P* | 5% E/P* |
| "Methoxyphytofluene" (II) | 8% B/P | 5% E/P |
| 3,4,11′,12′-Tetrahydrospheroidene (III) | 15% B/P | 10% E/P |
| 11′,12′-Dihydrospheroidene (IV) | 15% B/P | 10% E/P |
| 3,4,Dihydrospheroidene (V) | 15% A/P* | 10% E/P |
| Spheroidene (VI) | 15% A/P | 10% E/P |
| 3,4,Dihydroanhydrorhodovibrin (VII) | 30% A/P | 15% E/P |
| Anhydrorhodovibrin (VIII) | 30% A/P | 15% E/P |
| 3,4,3′,4′,7′,8′,11′,12′-Octahydrospirilloxanthin (IX) | 15% A/P | 25% E/P |
| 3,4,3′,4′,7′,8′,-Hexahydrospirilloxanthin (X) | 30% A/P | 30% E/P |
| 3′,4′,7′,8′-Tetrahydrospirilloxanthin (XI) | 30% A/P | 30% E/P |
| 3,4,3′,4′-Tetrahydrospirilloxanthin (XII) | 30% A/P | 30% E/P |
| 3,4-Dihydrospirilloxanthin (XIII) | 30% A/P | 40% E/P |
| Spirilloxanthin (XIV) | 40% A/P | 40% E/P |

* Abbreviations: A = acetone, B = benzene, E = diethyl ether, P = light petroleum, b.p. 40–60°.

### *Electronic and Mass Spectrometry*

Electronic spectra were determined in light petroleum (b.p. 40–60°). Mass spectra were determined on an A.E.I. MS 12 instrument, using the direct inlet system, and an ion source temperature of 240°.

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