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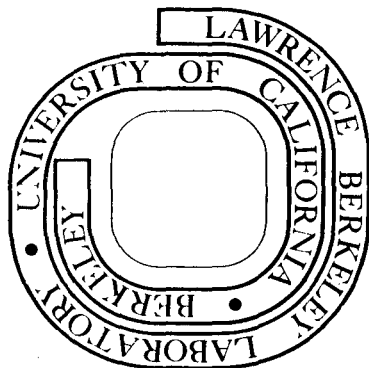
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ISOPRENOID HYDROCARBONS PRODUCED BY THERMAL ALTERATION OF
NOSTOC MUSCORUM AND RHODOPSEUDOMONAS SPHEROIDES

by

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ABSTRACT

Pure cultures of Nostoc muscorum and Rhodopseudomonas spheroides have been subjected to varying periods of thermal alteration. Experiments were conducted using both unextracted and extracted cultures in the absence and presence of montmorillonite. The isoprenoid hydrocarbons produced in these experiments have been examined. The major hydrocarbons produced being phytane and five isomeric phytenes. It appears that the relative rate of formation of phytane is greater from the unextracted residues compared to the extracted residues, suggesting structural differences in the phytyl side-chains of the chlorophylls responsible for the formation of these isoprenoid hydrocarbons.

INTRODUCTION

In the course of our work on examining the potential of algae and bacteria as precursors of kerogen (PHILP and CALVIN, 1976), it was necessary to subject pure cultures of certain organisms to varying periods of thermal alteration. The thermal alteration experiments, performed on cultures of Nostoc muscorum and Rhodospseudomonas spheroides, were designed to serve two purposes. First, to see whether or not kerogen formation could be simulated by thermal alteration of the organisms. Second, to determine the rate of formation of C₁₆-C₂₀ isoprenoid hydrocarbons by the organisms under varying conditions. It is this latter topic with which this paper is concerned. The results from the first part of the study will be presented elsewhere.

The origin and method of formation of pristane and phytane in crude oil has been the focus of many research projects in recent years. The unanimous conclusion from the studies is that pristane and phytane undoubtedly have their origin from the phytyl side-chain of chlorophyll. However, the exact mechanism of formation of these isoprenoid hydrocarbons has been a constant source of discussion and disagreement. A recent report, by IKAN and co-workers (1975), on this subject proposes that degradation of chlorophyll in a generally reducing environment would favour the formation of phytane via dihydrophytol; however, exposure to an oxidizing environment during early diagenesis would favour the formation of phytenic acids which would ultimately yield pristane.

Previous to this study, several other workers suggested that certain isoprenoid compounds may form as a result of lithification or thermal alteration of phytol in a sediment column. These include dihydrophytol (SEVER and PARKER, 1969), a C₁₈ isoprenoid ketone (6, 10, 14-trimethylpentadecane-2-one; IKAN

et al., 1973; SIMONEIT, 1973), and C₁₉ and C₂₀ isoprenoid alkanes. In another study, MAXWELL et al. (1972, 1973) and COX et al. (1972a) provided stereochemical evidence for the formation of phytane and intermediates from the phytol ester of chlorophyll at relatively low temperatures. As a result of this study they (COX, et al., 1972b) proposed a scheme for the transformation of phytol into C₁₈-C₂₀ isoprenoid alkanes.

In the present study, samples of a pure culture of Nostoc muscorum were heated for two, four, and twelve weeks in sealed glass tubes at 200°C. Three different sets of experiments were performed over each of these time periods. Firstly, the pure culture was heated alone; secondly, it was heated in the presence of montmorillonite; finally, the culture was exhaustively extracted with organic solvents and using this residue, the heating experiments were repeated in the absence of montmorillonite. In a separate set of experiments performed for comparative purposes, the photosynthetic bacterium, Rhodospseudomonas spheroides, was heated in the same way as above (with and without montmorillonite) except the set of experiments using extracted residues was omitted.

The above experiments were not designed to elucidate or propose another mechanism for the source or formation of pristane and phytane from chlorophyll. It is clear that in this set of experiments the source of any isoprenoid hydrocarbons is the algal chlorophyll. Rather, these experiments were designed to see what factors effect the rate of formation of the hydrocarbons. Were they preferentially formed from the unbound chlorophyll or the intra-cellular chlorophyll? Was their formation catalysed by the presence of a clay-mineral catalyst, montmorillonite? Could they be formed under relatively mild thermal conditions?

We now wish to report the answers to some of these questions. Our results show that, in general, increasing amounts of phytane are formed, in both of

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the organisms examined with increasing time, and the relative amounts of phytanes decrease. However, in the case of the extracted organisms, the rate of formation of phytane is relatively slower than with the unextracted organisms. Montmorillonite catalysed the relative rate of formation of phytane in the experiments with the Rhodopseudomonas spheroides, but in no experiment were any phytadienes, pristane or pristenes detected.

EXPERIMENTAL

One-gram samples of the pure cultures Nostoc muscorum and Rhodopseudomonas spheroides (HAN, 1970) were sealed, under nitrogen, in thick-walled glass tubes and subjected to heat treatment at 200°C for varying periods of time (two, four and twelve weeks). The experiments were repeated using the same organisms mixed with montmorillonite in the ratio 1 part organism to 2.5 parts montmorillonite. Finally, the experiments were repeated with samples of Nostoc muscorum which had been exhaustively extracted with toluene/methanol (1:1) for fourteen days to remove all the soluble lipid material. At the end of each experiment, the tubes were opened and the residues extracted with toluene/methanol (1:1) using ultra-sonication. The extracts were fractionated on an alumina column into heptane, ethyl acetate and methanol fractions. The heptane extracts were analysed by gas chromatography (GC) and computerized-gas chromatography-mass spectrometry (C-GC-MS).

All analytical gas-liquid chromatography (GC) was carried out on a Varian 2700 gas chromatograph. The total, normal and branched/cyclic fractions were chromatographed on a 20' x 0.03" i.d. glass column, packed with 3% Dexsil 300 on Gas Chrom Q, and temperature programmed from 70 to 280°C at 4°/min with a helium flow rate of 6 ml/min. (The same GC conditions were used throughout

the study). The various components of the mixtures analysed by GC were identified by their retention times, co-injection of standards, wherever possible, and low resolution mass spectra obtained by combined gas chromatography-mass spectrometry (GC-MS). Combined GC-MS analyses were carried out on a DuPont 492-1 mass spectrometer interfaced with a Varian Aerograph GC Model No. 204 equipped with linear temperature programmer. The column used for the GC-MS analyses was also a 20' x 0.03" i.d. glass capillary column packed with 3% Dexsil 300 on Gas Chrom Q. The mass spectral data were acquired and processed using a DuPont 21-094 data system.

(The insoluble organic residues from each set of experiments were set aside for characterization by elemental analyses, infra-red spectroscopy, transmitted and reflected light microscopy and SEM photography. The results from these characterizations will be reported in a separate paper.)

RESULTS AND DISCUSSION

Table I summarizes the yields of various fractions obtained from each set of experiments. The gas chromatograms obtained for the hydrocarbon fractions from each set of experiments are shown in Figs. 1-5. In each chromatogram the peaks which have been labelled with identical numbers have been shown by mass spectrometry to arise from the same compounds. The structural assignments made to these compounds are listed in Table II.

An examination of Table I shows that, in general, increasing amounts of hydrocarbons are produced from the residues with increasing time of heating. (Similarly, the amounts of ethyl acetate and methanol fractions also increase with time of heating.) There is not a great deal of difference between the quantities of hydrocarbons produced from the unextracted or the extracted

residues of Nostoc muscorum. However, in the presence of montmorillonite, the quantities produced are considerably higher. The yields of hydrocarbons from the unextracted culture of Nostoc muscorum are lower than those from the Rhodopseudomonas spheroides. (The yields of the ethyl acetate and methanol fractions are also higher for the unextracted Rhodopseudomonas spheroides compared to the Nostoc muscorum.)

Prior to discussing the qualitative nature of these results, it is important to note that the major hydrocarbons obtained from extraction of the pure culture of Nostoc muscorum were $n\text{-C}_{17}\text{H}_{36}$, a mixture of 7- and 8-methylheptadecane with minor amounts of the $n\text{-C}_{15}\text{H}_{32}$, $n\text{-C}_{16}\text{H}_{34}$ and $n\text{-C}_{18}\text{H}_{38}$ hydrocarbons. It is important to note that no pristane, phytane, phytenes, or phytadienes were detected in the soluble lipid extract, which agrees with the finding of HAN (1970).

From Fig. 1 and Table II it can be seen that with increasing time of heating, the unextracted Nostoc muscorum produces more phytane and relatively smaller amounts of the phytenes. (No pristane or phytadienes were formed in any of the experiments performed in this study.) The results from the extracted residue of Nostoc muscorum differ in two ways (Fig. 2). First, the $n\text{-C}_{17}\text{H}_{36}$ and 7- and 8-methylheptadecanes are absent. Second, the relative rate of formation of phytane in the first two time periods appears to be much slower. There are significant amounts of compounds, tentatively identified by C-GC-MS as being monounsaturated phytenes, present in the products from all three experiments, but it is only after twelve weeks that significant amounts of phytane can be detected. In the presence of montmorillonite, the relative rate of formation of phytane from the Nostoc muscorum in the two- and four-week heating periods appears to be reduced (cf. Fig. 3 with Fig. 1). No ready explanation can be given for this observation at this time.

In the two sets of experiments performed on the Rhodopseudomonas spheroides similar trends were again observed. In the two- and four-week experiments with the unextracted residues, phytene is the major product (Fig. 4). After twelve weeks phytane is the dominant product. In the presence of montmorillonite the amount of phytane relative to the phytene does not appear to be significant after four weeks, as can be seen in Fig. 5, but after twelve weeks the products are again dominated by phytane.

Why are there differences between the rates of formation of these hydrocarbons from the unextracted and extracted residues of both organisms, especially in the initial stages of heating? It would appear that in the unextracted residues, the phytane and the phytene are formed from the unbound chlorophyll in these samples. After exhaustive extraction of the residues, the only source of the hydrocarbons is the intracellular chlorophyll. In the unextracted sample the presence of free phytol could also increase the rate of appearance of the phytane, but in the extracted samples there is no free phytol present prior to thermal alteration of the residues. Therefore, the rate of phytane formation will be slower in the latter case since cleavage of the phytol side-chain of chlorophyll has to occur prior to formation of the isoprenoid hydrocarbons.

The $n\text{-C}_{17}\text{H}_{36}$ and 7- and 8-methylheptadecanes would not be expected to be present in the products from the experiments involving the extracted residues. These compounds are removed from the residues during the solvent extraction process and are not formed by thermal alteration of the residue.

The absence of pristane is interesting and could imply that it is not necessarily formed under the same conditions as phytane. However, if as IKAN et al. (1975) suggest, pristane does form from phytol via phytanic acid and pristene, it would appear that the rate of formation under the tempera-

ture conditions in this study is insignificant. It is also possible that the pristane found in the study by IKAN et al. (1975) was not formed directly from chlorophyll by the suggested pathway but had an alternative, and as yet unknown, source. On the other hand, BAYLISS (1968) subjected chlorophyll from algae and alfalfa to hydrogenolysis at 800°F in a hydrogen atmosphere. Under these conditions the ratio of phytane to pristane from the algal chlorophyll was about 3:1. These results, along with our observations, would suggest that the formation of phytane via phytol, dihydrophytol and phytene is a relatively mild process but to form pristane, more severe heating conditions for longer periods of time are required.

BROOKS et al. (1969) suggested that variations in the pristane:phytane ratio in coals and crude oils may reflect variations in oxidation during earliest stages of chlorophyll decomposition. They proposed that a low pristane:phytane ratio in a crude oil may be an indication of marine plant origin. The reason for this is that the formation of phytanic acid, the precursor of pristane, is less likely in the more anaerobic conditions in aquatic environments than it is on land during decay of plant material. Our results support this theory since no detectable amounts of pristane were formed during our heating experiments which were performed under an absence of oxygen.

It is noteworthy to mention that LIJMBACH (1975) has also performed similar heating experiments on mixed cultures of bacteria both before and after extraction with chloroform. These experiments were performed in an attempt to support the theory that the "non-lipid" part of bacterial and algal bodies have contributed to a large extent in the formation of crude oil. However, the saturate fractions obtained from Lijmbach's work appear to be dominated by n-alkanes with neither pristane or phytane being formed by heating of the mixed cultures of bacteria.

CONCLUSION

In all five sets of experiments the amount of phytane formed relative to the phytenes increases with time. In the case of the Nostoc muscorum the relative rate of phytane formation was slower in the experiments using the exhaustively extracted residues. This observation suggests that there are fundamental differences between the molecular structures of the phytyl component in the "free" and intracellular chlorophyll. Alternatively, the presence of free phytol in the unextracted residue could increase the rate of phytane formation. The presence of montmorillonite appears to enhance the formation of the phytenes but not the relative rate of formation of the phytane.

The distribution and types of products formed from both the Nostoc muscorum and the Rhodopseudomonas spheroides experiments were similar, supporting the theory that they have a common origin from the chlorophyll moiety. The absence of pristane, pristenes and phytadienes from any of these experiments is extremely interesting. The only theory for this observation at the present time is that the relatively mild temperatures used precluded their formation within the time limits of this study.

In conclusion, therefore, this study has shown that the relative rates of formation of phytane and phytenes will vary depending on the source of the chlorophyll responsible for its formation.

ACKNOWLEDGMENTS

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REFERENCES

- BAYLISS, G. S. (1968) The formation of pristane, phytane and related isoprenoid hydrocarbons by the thermal degradation of chlorophyll. Amer. Chem. Soc. Abstr. Papers 155, J-12.
- BROOKS, J. D., GOULD, K. and SMITH, J. W. (1969) Isoprenoid hydrocarbons in coal and petroleum. Nature 222, 257-259.
- COX, R. E., MAXWELL, J. R., ACKMAN, R. G. and HOOPER, S. N. (1972a) Stereochemical studies of acyclic isoprenoid compounds - III. The stereochemistry of naturally occurring (marine) 2, 6, 10, 14-tetramethylpentadecane. Can. J. Biochem. 50, 1238-1241.
- COX, R. E., MAXWELL, J. R., ACKMAN, R. G. and HOOPER, S. N. (1972b) The isolation of a series of acyclic isoprenoid alcohols from an ancient sediment: approaches to a study of the diagenesis and maturation of phytol. In: Advances in Organic Geochemistry, (editors H. R. von Gaertner and H. Wehner), pp. 263-276. Pergamon Press.
- HAN, J. (1970) Chemical studies of terrestrial and extraterrestrial life. Ph.D. thesis, U. of California, Berkeley.
- IKAN, R., BAEDECKER, M. J. and KAPLAN, I. R. (1973) C₁₈-Isoprenoid ketone in recent marine sediment. Nature 244, 154-155.
- IKAN, R., BAEDECKER, M. J. and KAPLAN, I. R. (1975) Thermal alteration experiments on organic matter in recent marine sediments - II. Isoprenoids. Geochim. Cosmochim. Acta 39, 187-194.
- LIJNBACH, G. W. M. (1975) On the origin of petroleum. Proc. Ninth World Petrol. Cong. 2, 357-369.
- MAXWELL, J. R., COX, R. E., ACKMAN, R. G. and HOOPER, S. N. (1972) The diagenesis and maturation of phytol. The stereochemistry of 2, 6, 10, 14-tetramethylpentadecane from an ancient sediment. In: Advances in Organic Geochemistry, (editors H. R. von Gaertner and H. Wehner), pp. 277-291. Pergamon Press.
- MAXWELL, J. R., COX, R. E., EGLINTON, G. and PILLINGER, C. T. (1973) Stereochemical studies of acyclic isoprenoid compounds - II: The role of chlorophyll in the derivation of isoprenoid-type acids in a lacustrine sediment. Geochim. Cosmochim. Acta 37, 297-313.
- PHILIP, R. P. and CALVIN, M. (1976) Possible origin for insoluble organic (kerogen) debris in sediments from insoluble cell-wall materials of algae and bacteria. Nature 262, 134-136.
- SEVER, J. and PARKER, P. L. (1969) Fatty alcohols (normal and isoprenoid) in sediments. Science 164, 1052-1054.

Table I

YIELDS OF SOLUBLE PRODUCTS FROM IN THERMAL ALTERATION EXPERIMENTS

RESIDUE	HEATING PERIOD	WT. OF RESIDUE USED		WTS. OF FRACTIONS FROM COLUMN CHROMATOGRAPHY ¹					
		A	B	Heptane		Ethyl Acetate		Methanol	
				A	B	A	B	A	B
Pure Culture	2 weeks	1 g	1 g	0.3	2.5	24.3	137.6	178.0	381.5
	4 weeks	1 g	1 g	0.4	1.5	51.5	137.2	190.0	435.8
	12 weeks	1 g	1 g	0.8	20.0	63.6	142.2	182.3	137.0
Extracted Culture ²	2 weeks	1 g	-	0.2	-	34.0	-	301.1	-
	4 weeks	1 g	-	0.3	-	96.7	-	373.3	-
	12 weeks	1 g	-	0.2	-	147.2	-	355.5	-
Pure Culture + Montmorillonite (MM)	2 weeks	1 g A +2.5 g MM	1 g B +2.5 g MM	0.8	1.8	24.5	214.5	35.6	233.7
	4 weeks	1 g A +2.5 g MM	1 g B +2.5 g MM	1.5	3.3	90.5	259.6	165.1	259.4
	12 weeks	1 g A +2.5 g MM	1 g B +2.5 g MM	13.1	3.0	146.6	76.0	210.5	178.0

A Nostoc muscorumB Rhodopseudomonas spheroides

1 In mgs.

2 This set of experiments not attempted with Rhodopseudomonas spheroides

Table II

IDENTITY OF MAJOR HYDROCARBON COMPONENTS

Peak No.*	Molecular Formula	Structural Identity
1	$C_{17}H_{36}$	n-C ₁₇ alkene
2	$C_{18}H_{38}$	7- and 8-methylheptadecane
3	$C_{20}H_{40}$	Phytene**
4	$C_{20}H_{42}$	Phytane
5	$C_{20}H_{40}$	Phytene**
6	$C_{20}H_{40}$	Phytene**
7	$C_{20}H_{40}$	Phytene**
8	$C_{20}H_{40}$	Phytene**

* These numbers correspond to numbers of peaks in Figs. 1-5.

** These five components have been tentatively identified by C-GC-MS as being monounsaturated phytene, although the exact position of the double bond has not been established.

Fig. 1 Hydrocarbons produced by thermal alteration of non-extracted Nostoc muscorum. Identities of components responsible for peaks 1-8 can be found in Table II. GC conditions as stated in text.

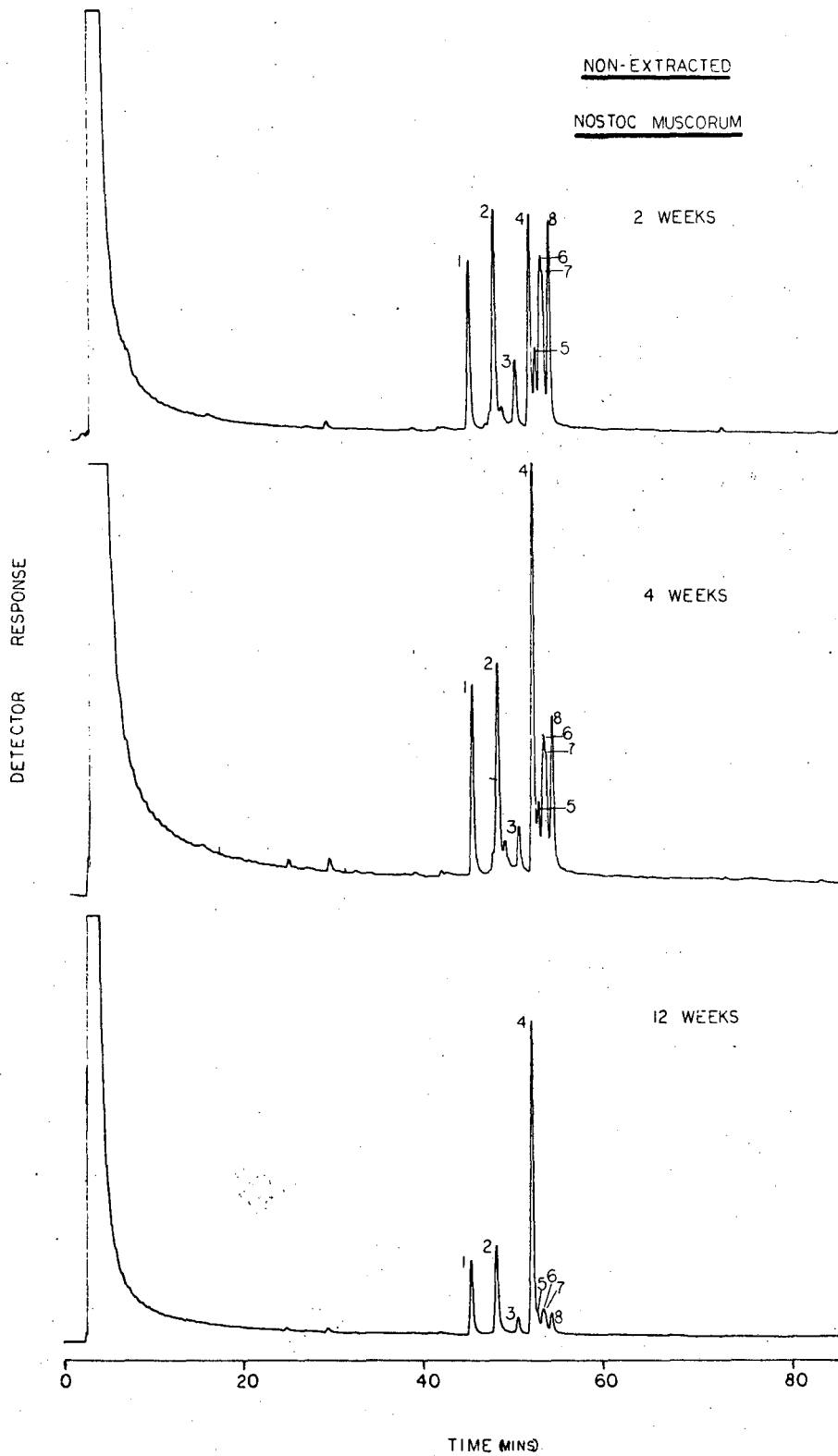


Fig. 2 Hydrocarbons produced by thermal alteration of extracted Nostoc muscorum. Identities of components responsible for peaks 1-8 can be found in Table II. GC conditions as stated in text.

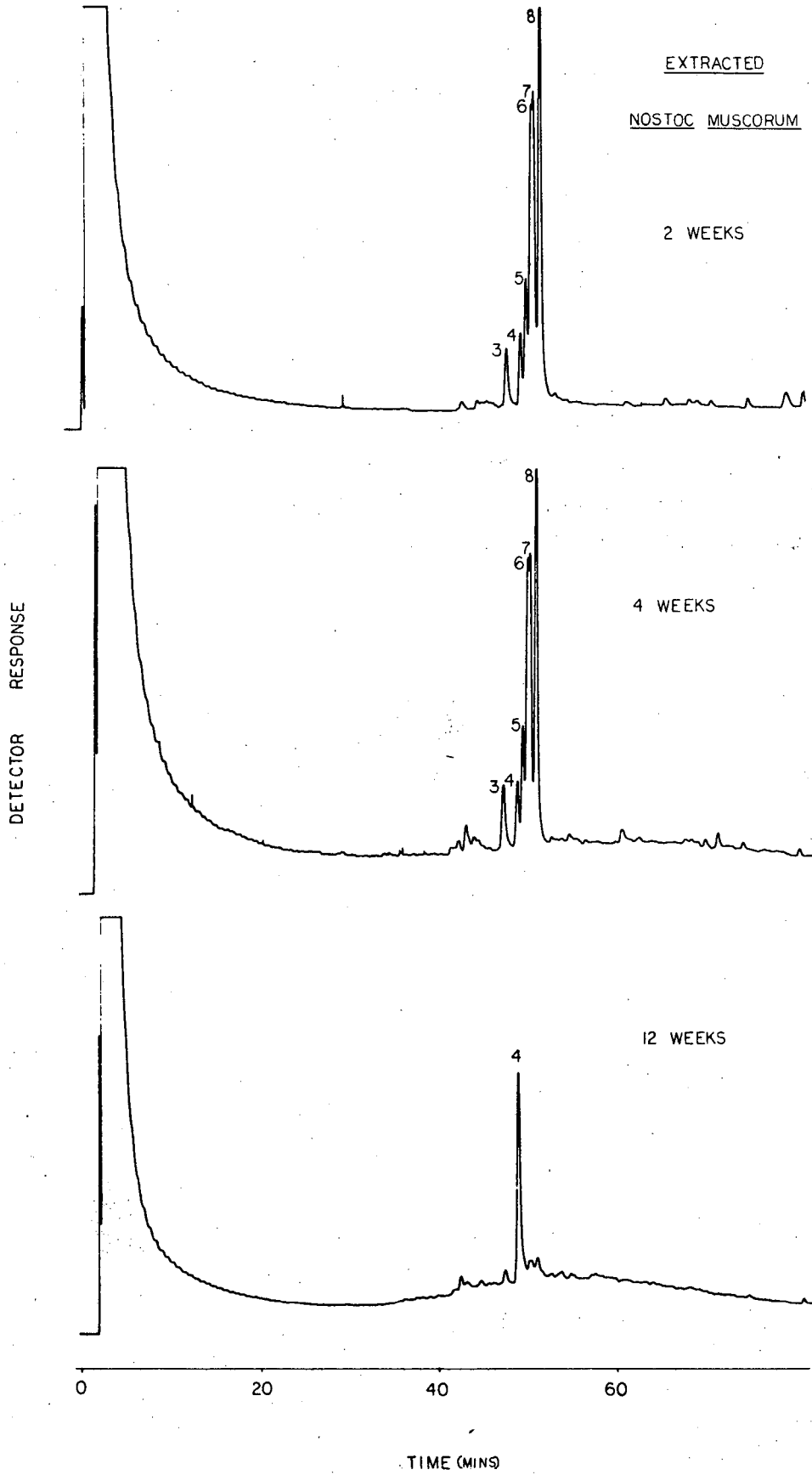


Fig. 3 Hydrocarbons produced by thermal alteration of non-extracted Nostoc muscorum + montmorillonite. Identities of components responsible for peaks 1-8 can be found in Table II. GC conditions as stated in text.

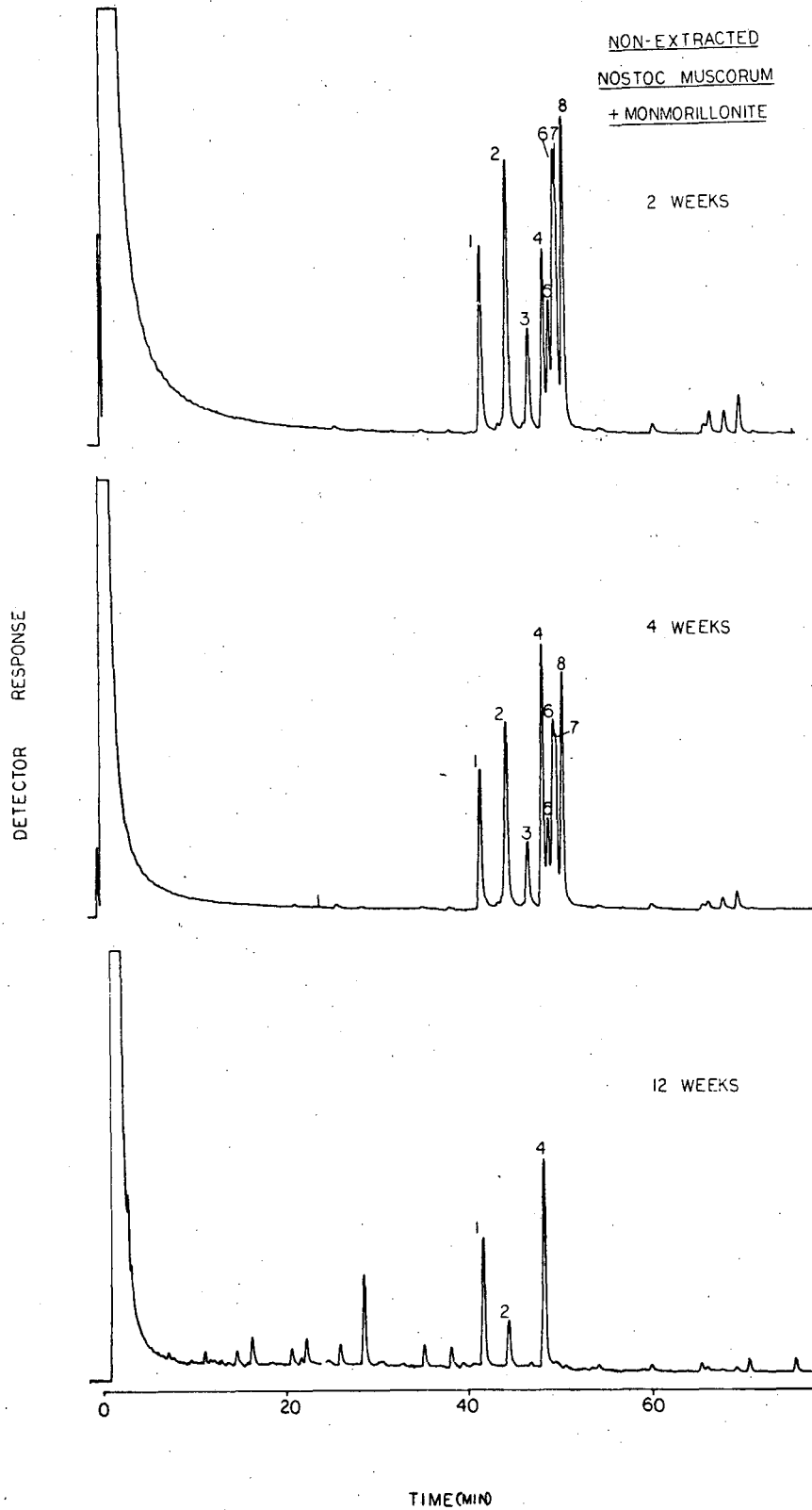


Fig. 4 Hydrocarbons produced by thermal alteration of non-extracted Rhodopseudomonas spheroides. Identities of components responsible for peaks 1-8 can be found in Table II. GC conditions as stated in text.

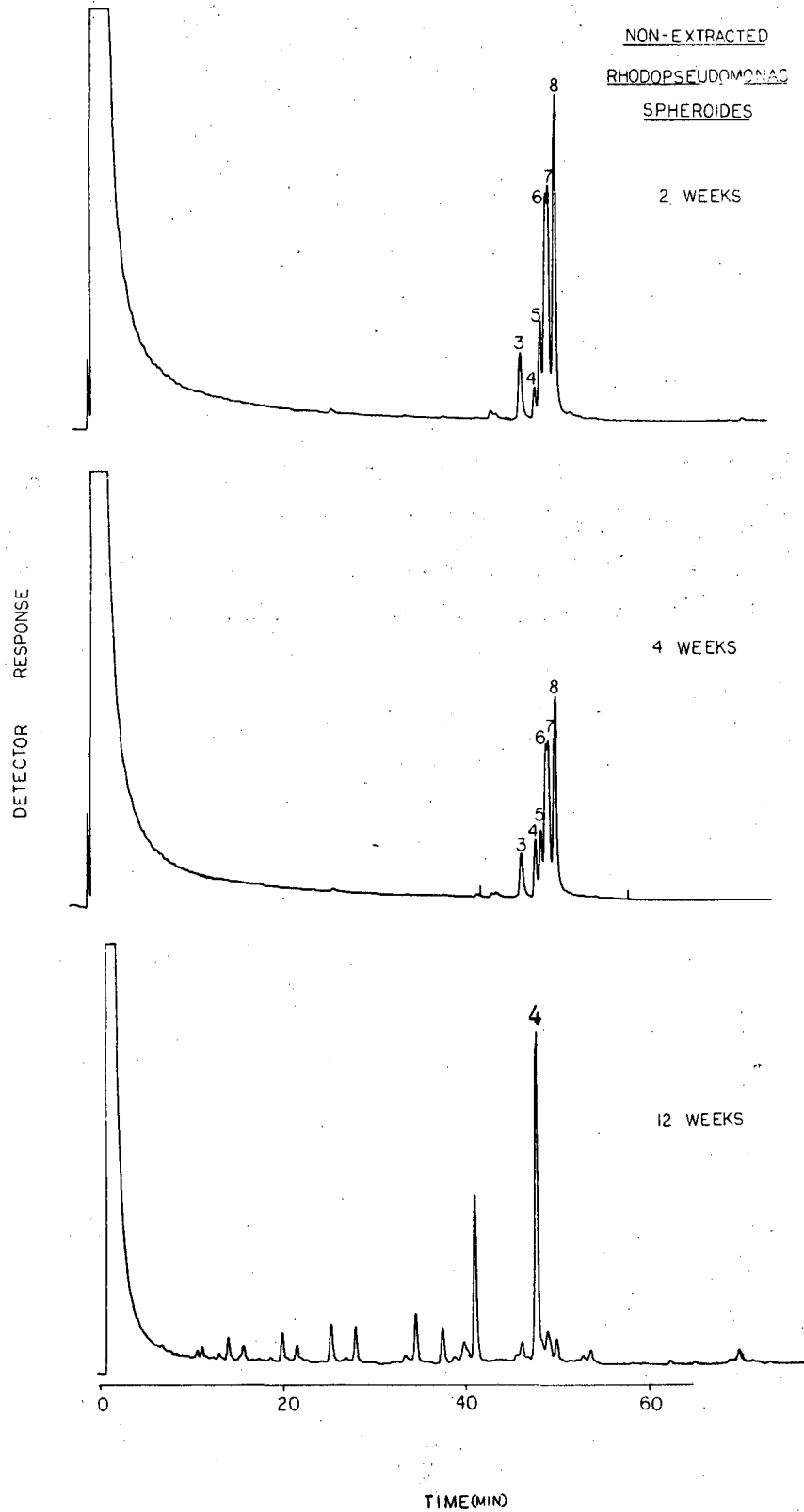
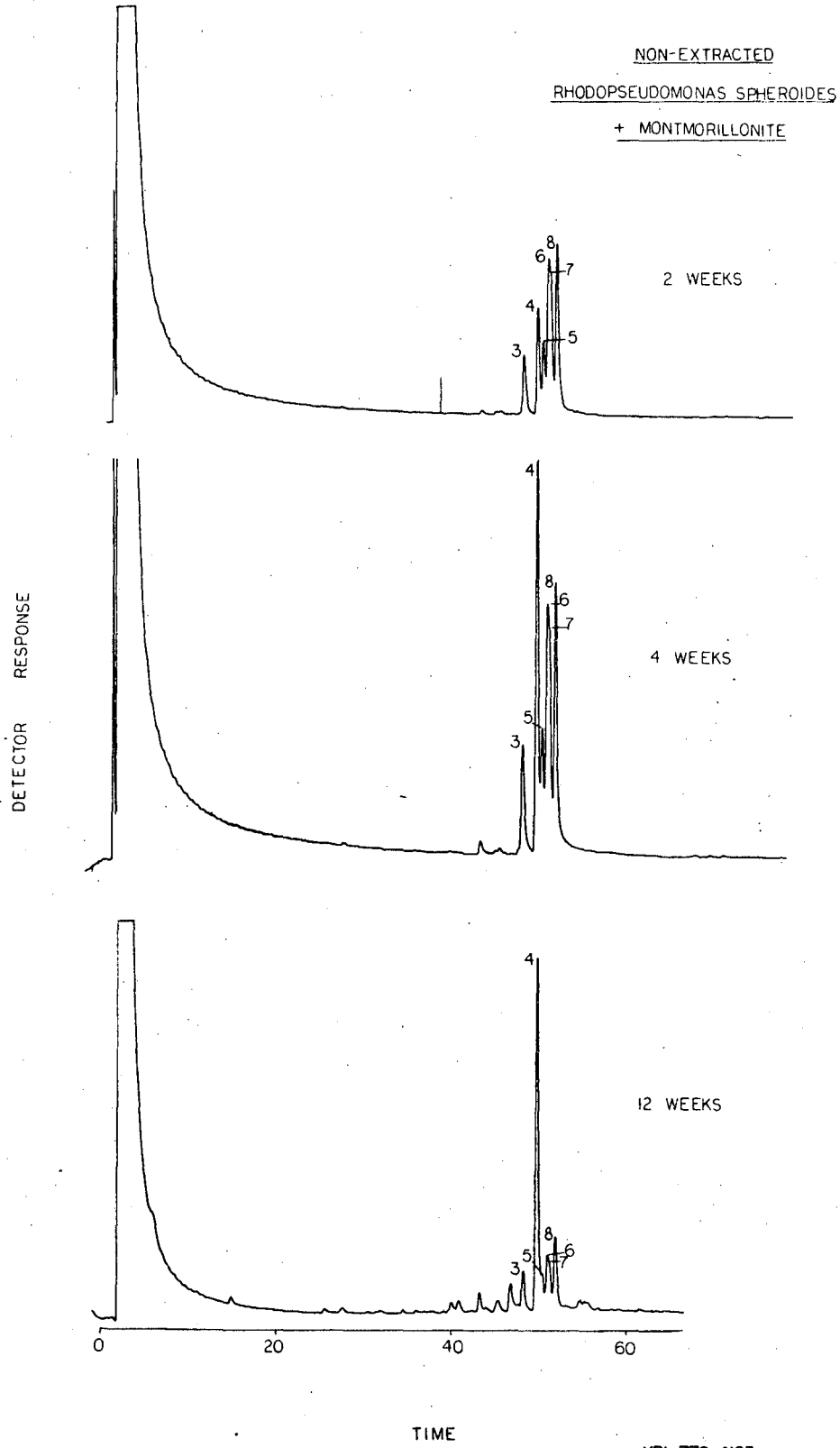


Fig. 5 Hydrocarbons produced by thermal alteration of non-extracted Rhodopseudomonas spheroides + montmorillonite. Identities of components responsible for peaks 1-8 can be found in Table II. GC conditions as stated in text.



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