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Novel long-chain *anteiso*-alkanes and *anteiso*-alkanoic acids in Antarctic rocks colonized by living and fossil cryptoendolithic microorganisms

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ABSTRACT

Saponified extracts of rock samples colonized by cryptoendolithic microbial communities from the McMurdo Dry Valleys of Southern Victoria Land, Antarctica, were separated into hydrocarbon and fatty acid fractions by silica gel column chromatography. Hydrocarbons and methyl esters of fatty acids were analyzed by capillary gas chromatography–mass spectrometry. Unusually, a suite of long-chain *anteiso*-alkanes (a-C_{20} to a-C_{30}) and *anteiso*-alkanoic acids (a-C_{20} to a-C_{30}) were detected in many samples, together with straight-chain, branched and/or cyclic and acyclic isoprenoid compounds. These novel compounds are probably derived from unidentified heterotrophic bacteria or symbiotic processes in a unique microbial community in the Antarctic cold desert and suggest the occurrence of a special biosynthetic pathway. Long-chain *anteiso*-alkanes are probably formed through microbial decarboxylation of corresponding *anteiso*-alkanoic acids. They may serve as new biomarkers in environmental and geochemical studies.

INTRODUCTION

Most of Antarctica is covered by an ice sheet approximately 2450 m thick, but ice-free areas are sparsely distributed in coastal regions and inland mountains. The Ross Desert (the McMurdo Dry Valleys) of Southern Victoria Land is the largest ice-free area in Antarctica and covers 2500 km^2. The Ross Desert environment is extremely cold and dry, and the surface is virtually abiotic. Cryptoendolithic microbial communities colonize the near-surface layers of porous rocks, where the rock is warmed by insolation to temperatures above the ambient [1–3]. Several distinct cryptoendolithic microbial communities exist. The most common ones

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are the lichen-dominated communities consisting of lichen-forming fungi and green algae, parasymbiotic fungi, free-living fungi and green algae and cyanobacteria, the red *Gloeocapsa* communities (several *Gloeocapsa* species and other cyanobacteria) and the *Hormathonema–Gloeocapsa* communities (formed by several cyanobacteria). All these communities harbor a number of (largely unidentified) heterotrophic bacteria [4]. In the Ross desert, organisms exist near the temperature limit below which life is impossible, and even slight changes in the environment result in death and extinction [5]. Communities in various stages of fossilization occur [6] and are common on Mt. Fleming (unpublished results), which is partly surrounded by the Antarctic ice sheet.

Hydrocarbons and fatty acids are two of the most widely distributed groups of organic compounds in living organisms and natural environments. In geological settings from the Holecene to the Precambrian, these compounds can be used to evaluate sources, maturation, alteration and sedimentary conditions of organic matter in various environments [7–10]. Long-chain *n*-alkanes ($>C_{19}$) with a predominance of odd carbon numbers are believed to originate from the waxes of vascular plants. *Iso*- and/or *anteiso*-alkanes are reported to occur in ancient sediments [11], coals [12] and air–sea interface samples [13]. Their proposed sources are the waxes of vascular plants and/or bacteria [11,12]. Short-chain *n*-alkanoic acids ($<C_{20}$) occur in most living organisms, and long-chain *n*-alkanoic acids (*n*-C_{20} to *n*-C_{34}) are abundant in the waxes of vascular plants [14,15]. Although short-chain *iso*- and *anteiso*-alkanoic acids are major lipids of certain bacteria [16], little is known about the occurrence of long-chain *anteiso*-alkanoic acids ($>C_{19}$) in the natural environment. Here we report capillary gas chromatographic–mass spectrometric (GC–MS) results of the discovery of a series of novel long-chain *anteiso*-alkanes (*a*-C_{20} to *a*-C_{30}) and long-chain *anteiso*-alkanoic acids (*a*-C_{20} to *a*-C_{30}) in rock samples colonized by cryptoendolithic microbial communities of the Ross Desert in Southern Victoria Land, Antarctica, and discuss their possible sources and geochemical and biological significance.

EXPERIMENTAL

Apparatus and chemicals

Care was taken to avoid contamination of samples; glassware rather than plastics was used throughout. Liquid chromatographic-grade organic solvents [hexane, benzene and ethyl acetate (Wako)] were used without any treatment. Silica gel (100 mesh) (Mallinckrodt) was used after heating at 500°C for 2 h to remove organic contaminants. Platinum dioxide (Wako) was used without any treatment. Boron-trifluoride methanol (51:49) (GL Science) was diluted to 14:86 with methanol. Authentic alkanes and alkanoic acids (99%) were purchased from GL Science. The GC–MS measurements were carried out with Shimadzu GCMS QP1000 gas chromatograph–mass spectrometer equipped with a cooled on-column injector.

Sampling sites and samples

The Beacon Supergroup, a Gondwanaland sediment, mainly composed of sandstone, forms the dominant rock type at higher elevations throughout the region [17]. It provides a favorable habitat for cryptoendolithic microorganisms. Rock (sandstone) samples colonized by cryptoendolithic microbial communities were collected from Linnaeus Terrace (77°36′S, 161°05′E, elevation 1600–1650 m) in the Asgard Range and Mount Fleming (77°33′S, 160°07′E, elevation 2200 m) and other localities in the Ross Desert of Southern Victoria Land, Antarctica, during the austral summers of 1977–1986 by E.I.F. and R.O.-F. All samples were kept frozen (−30°C) until analyzed in 1988–1991 by G.I.M. and K.W. The sandstone samples were crushed into particles of the original sand size in an agate mortar.

Analytical procedures

The analytical methods for hydrocarbons and fatty acids have been described elsewhere [18]. Briefly, the crushed rock samples (10–50 g) were refluxed with 0.5 *M* potassium hydroxide in methanol (80°C, 2 h) and extracted with ethyl acetate after acidification. Hydrocarbon and fatty acid fractions were obtained through a silica gel column (160 × 5 mm I.D., 100 mesh, 5% water). Fatty acids were methylated with boron trifluoride–methanol (14:86) (80°C, 2 h). Normal alkenes were identified after hydrogenation with hydrogen and platinum dioxide in hexane [19]. Hydrocarbons and fatty acids were analyzed by GC–MS.

GC–MS was operated with a DB-5 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm) (J&W Scientific). The column temperature was programmed from 70 to 120°C at 25°C/min and then from 120 to 310°C at 6°C/min. Fatty acids were analyzed by GC–MS with a DB-225 fused-silica capillary column (J&W Scientific) of the same size as the DB-5 column to resolve the degree of unsaturation of the alkenoic acids. The columm temperature was programmed from 70 to 120°C at 25°C/min and then from 120 to 240°C at 5°C/min. The molecular separator and ion source were maintained at 320 and 250°C, respectively. The flow-rate of helium carrier gas was 4.3 ml/min. Mass spectra (m/z 50–600) were taken continuously at intervals of 1.3 s at 70 eV. The hydrocarbons and fatty acid methyl esters were identified by comparision of the retention sequences and mass spectra with those of authentic compounds and published data [18–21]. The determination of hydrocarbons and fatty acids was performed by measurement of peak heights on the gas chromatograms (TIC) and/or mass chromatograms (m/z 71 for alkanes; m/z 74 for alkanoic acids).

RESULTS AND DISCUSSION

Identification

A series of long-chain *anteiso*-alkanes from *a*-C_{20} to *a*-C_{30}, with a peak at *a*-C_{26}, were found in the capillary gas chromatogram of the hydrocarbon fraction of a rock sample colonized by cryptoendolithic lichen-dominated microbial communities from the Ross Desert of Antarctica, toghether with a suite of *n*-alkanes (*n*-C_{15} to *n*-C_{36}), isoprenoid alkanes (pristane and phytane) and/or *n*-$C_{17:1,2}$ alkenes (Fig. 1). The mass spectra of *anteiso*-alkanes showed a strong peak at $M^+ - 29$ duo to α-cleavage, whereas those of *iso*-alkanes revealed a strong peak at $M^+ - 15$ (Fig. 2 [20]). Thus *anteiso*-alkanes are easily distinguished from *n*- and *iso*-alkanes and can be identified.

A series of *n*-alkanoic acids are detected in the

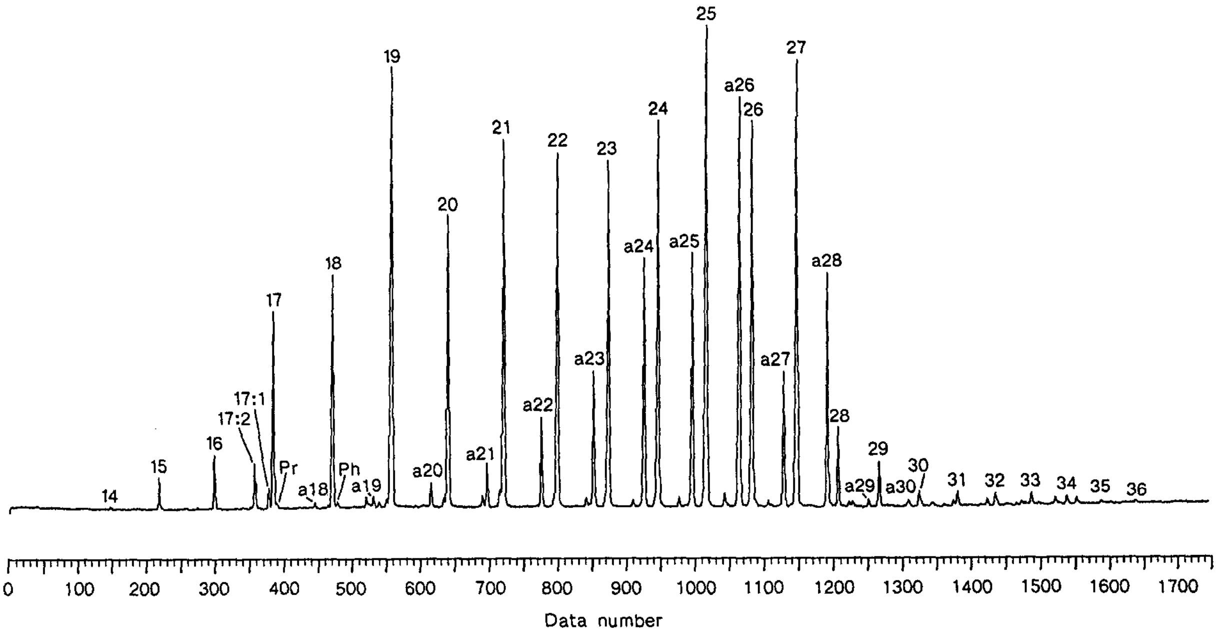

Fig. 1. Capillary gas chromatogram of the hydrocarbon fraction from a rock sample colonized by cryptoendolithic lichen-dominated microbial communities of the Ross Desert in Southern Victoria Land, Antarctica (A856-100). Arabic figures on the peaks denote carbon chain length of *n*-alkanes. Pr, Ph, and a are pristane, phytane and *anteiso*-alkanes, respectively. m:n = carbon chain length: number of double bonds.

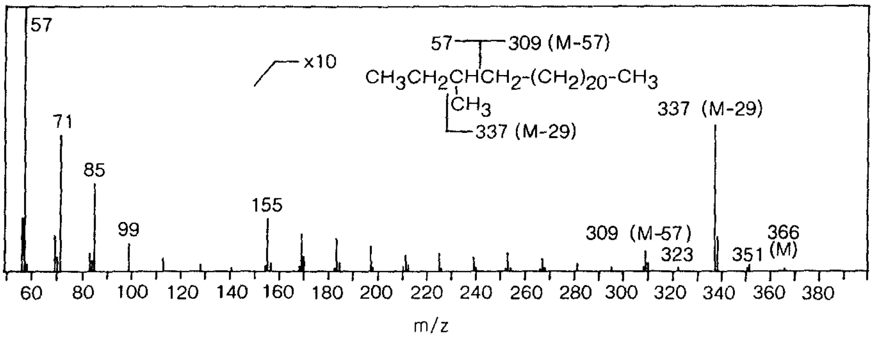

Fig. 2. Mass spectrum of long-chain *anteiso*-$C_{26:0}$ alkane found in a rock sample colonized by cryptoendolithic lichen-dominated microbial communities, Antarctica (A856-100).

capillary gas chromatogram of the fatty acid fraction of the above rock sample, together with short-chain *iso*- and *anteiso*-alkanoic and *n*-alkenoic acids (Fig. 3). Of special interest is the occurrence of a series of long-chain unidentified compounds. The mass spectra of these unknown compounds were similar to those of *n*-alkanoic acid methyl esters, so they probably cannot be distinguished from long-chain *n*- and/or *iso*-alkanoic acid methyl esters by mass spectra alone (Fig. 4). However, the *m/z* 57

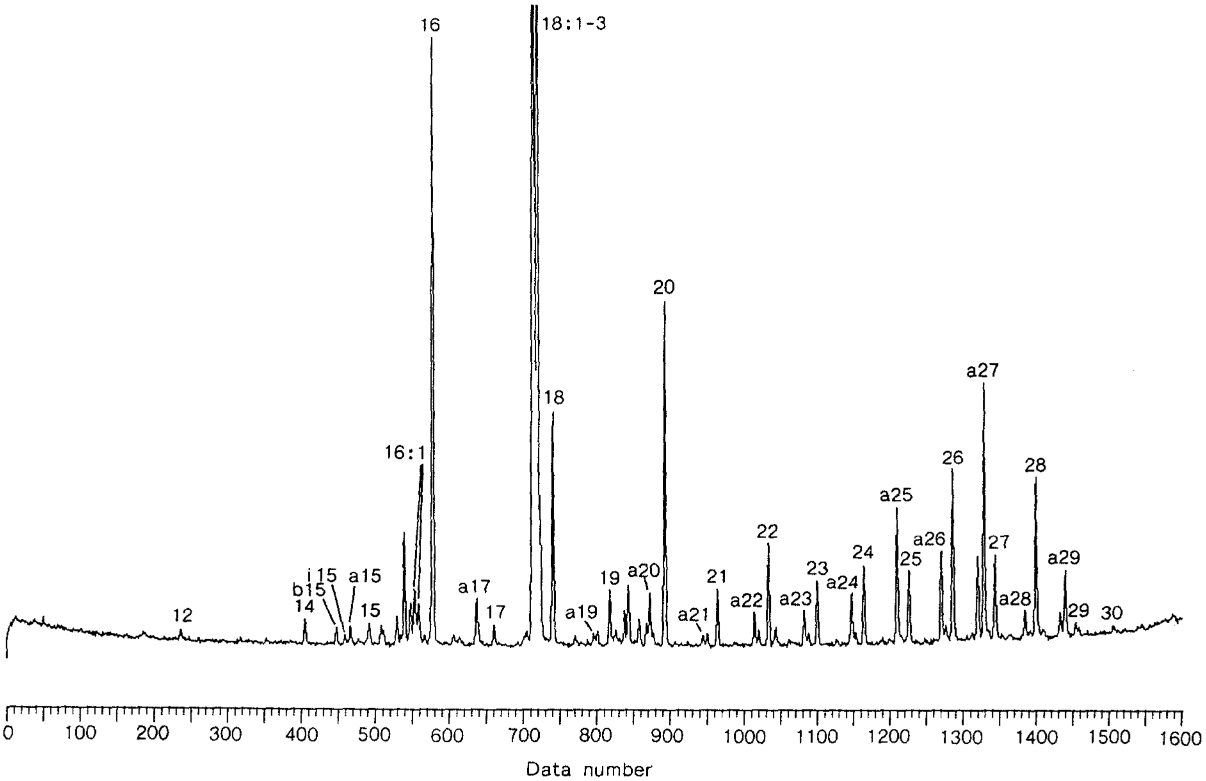

Fig. 3. Capillary gas chromatogram of the fatty acid fraction from a rock sample colonized by cryptoendolithic lichen-dominated microbial communities, Antarctica (A856-100). Arabic figures on the peaks denote carbon chain length of *n*-alkanoic acids; i and a are *iso*- and *anteiso*- alkanoic acids, respectively. *m*:*n* = carbon chain length: number of double bonds.

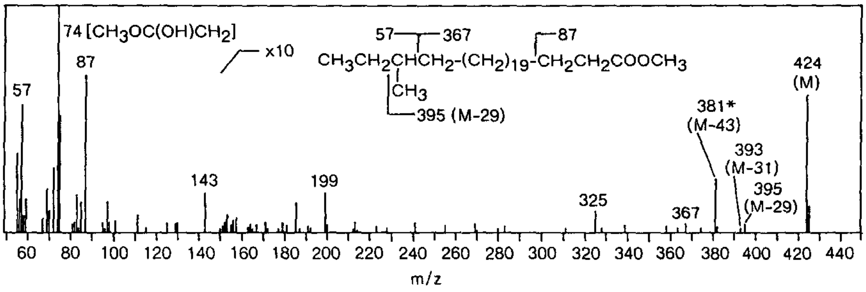

Fig. 4. Mass spectrum of the methyl ester of long-chain *anteiso*-$C_{27:0}$ alkanoic acid found in a rock sample colonized by cryptoendolithic lichen-dominated microbial communities, Antarctica (A856-100). The peak marked with an asterisk may be due to loss of a propyl group.

intensities of the unknown compounds are approximately 1.61 ± 0.15 (standard deviation) times greater than those of *n*-alkanoic acid methyl esters. The excess m/z 57 peak may be due to α-cleavage of methyl branch at the *anteiso*-position of the unknown compounds (Fig. 4). Equivalent carbon-chain length (ECL) values for the DB-5 column were also calculated from *iso*- and *anteiso*-C_{12}–C_{19} acid methyl esters found in the same chromatograms. The ECL values of *anteiso*-alkanoic acid methyl esters (n-C_{n-1} + 0.73 ± 0.01) were much greater than those of *iso*-alkanoic acid methyl esters (n-C_{n-1} + 0.63 ± 0.01). Generally the ECL values of a series of these unidentified peaks fit within the *anteiso*-alkanoic acid methyl esters (Table II). Also, a series of long-chain *anteiso*-alkanes with the same range were detected in these samples. These compounds were therefore identified to be long-chain *anteiso*-alkanoic acids.

Characteristics of hydrocarbons and fatty acids

Long-chain *anteiso*-alkanes were found in 40% of the 55 rock samples analyzed. Long-chain *anteiso*-alkanes were detected in some lichen-dominanted communities (*e.g.*, A856-100), fossils of lichen-dominated communities (*e.g.*, A867-110, A834-576, A845-523 and A856-82f), red *Gloeocapsa* communities and *Hormathonema–Gloeocapsa* communities, but were not found in any pure lichen communities, algal and cyanobacterial communities or uncolonized samples. The analytical results for long-chain *anteiso*-alkanes in five selected typical samples are shown in Table I, together with those for normal and isoprenoid hydrocarbons. Long-chain *anteiso*-alkanes are major hydrocarbons in the A856-100, A834-576, A845-523 and A856-82f samples (20.44–25. 95%). The predominant long-chain *anteiso*-alkane was a-C_{26} in all these samples (*e.g.*, Table I). Even-carbon-numbered long-chain *anteiso*-alkanes were predominant, as shown by the low odd/even carbon ratios (0.52–0.78). No one has reported such hydrocarbon distributions anywhere in the world. Interestingly, long-chain *n*-alkanes, were abundant in these samples (40.38–78.33%), although their odd/even carbon ratios were near unity (0.99–1.6).

Long-chain *anteiso*-alkanoic acids were found in 51% of the 55 rock samples. The analytical results for long-chain *anteiso*-alkanoic acids in five selected samples are shown in Table II. These samples contained *n*-alkanoic (n-C_9 to n-C_{32}), *n*-alkenoic (u-C_{16} to u-C_{18}) and short-chain *iso*- and *anteiso*- alkanoic acids (i,a-C_{12} to i,a-C_{18}), in addition to unidentified branched acids (b-C_{15} to b-C_{19}). A series of long-chain *anteiso*-alkanoic acids from a-C_{20} to a-C_{30} were detected in rock samples colonized by the lichen-dominated communities, the red *Gloeocapsa* communities and the *Hormathonema–Gloeocapsa* communities, and also in rocks containing fossil lichen-dominated communities, but were not detected in any pure lichen communities or in uncolonized rocks. Generally, long-chain *anteiso*-alkanoic acids were detected together with long-chain *anteiso*-alkanes. The most predominant *anteiso*-alkanoic acids was a-C_{27} in all samples (*e.g.*, Table II). Odd-carbon-numbered long-chain *anteiso*-alkanoic acids were predominant in all samples, as shown by the low even/odd carbon ratios (0.19–0.92). In addition, long-chain *anteiso*-alkanes are abundant acids in all samples shown in Table II (21.55–35.78%).

The total concentration of hydrocarbons and fat-

TABLE 1

HYDROCARBON COMPOSITIONS AND CONTENTS FOR ROCK SAMPLES COLONIZED BY CRYPTOENDOLITHIC LICHEN-DOMINATED MICROBIAL COMMUNITIES, ANTARCTICA

The lichen-dominated microbial communities consist of lichen-forming fungi and green algae, parasymbiotic fungi, free-living fungi and green algae, cyanobacteria and heterotrophic bacteria.

Composition (%)[a]	Linnaeus Terrace		Mount Fleming			
	A856-100[b]	A867-110[c]	A834-576[c]	A845-523[c]	A856-82f[c]	ECL[d]
n-Alkane (short)	12.16	10.38	8.14	10.14	16.09	
12:0	0.00	0.00	0.00	0.15	0.77	–
13:0	0.14	0.00	0.12	0.23	2.98	–
14:0	0.16	0.00	0.24	0.29	2.33	–
15:0	0.69	0.15	0.49	0.30	0.74	–
16:0	0.82	0.56	0.57	0.51	0.79	–
17:0	2.40	1.88	1.09	1.42	1.84	–
18:0	2.34	2.81	1.71	2.11	1.77	–
19:0	5.61	4.98	3.92	5.13	4.87	–
n-Alkane (long)	48.15	78.33	60.86	63.53	40.38	
20:0	3.11	7.32	3.08	3.98	2.38	–
21:0	4.22	9.89	4.48	5.13	3.12	–
22:0	4.69	11.22	5.80	5.28	3.00	–
23:0	5.75	11.03	7.65	6.94	3.87	–
24:0	6.11	10.84	6.20	7.70	4.86	–
25:0	7.75	9.28	8.04	9.29	5.89	–
26:0	6.05	5.99	7.57	8.45	5.59	–
27:0	7.00	7.30	9.85	9.21	6.20	–
28:0	1.58	1.52	2.92	2.79	2.14	–
29:0	0.95	1.24	2.12	1.86	1.35	–
30:0	0.28	0.72	0.81	0.60	0.41	–
31:0	0.23	0.71	0.65	0.51	0.41	–
32:0	0.21	0.48	0.59	0.74	0.41	–
33:0	0.14	0.37	0.45	0.51	0.32	–
34:0	0.09	0.32	0.40	0.35	0.29	–
35:0	0.00	0.10	0.13	0.11	0.15	–
36:0	0.00	0.00	0.11	0.06	0.00	–
n-Alkene	3.00	0.28	7.25	0.45	0.59	
17:1	1.51	0.00	0.49	0.00	0.00	–
17:2	1.49	0.22	6.76	0.45	0.59	–
Anteiso	25.95	9.56	21.61	20.44	23.66	
20:0	0.43	0.00	0.30	0.34	0.37	19.71
21:0	0.69	0.00	0.35	0.54	0.68	20.71
22:0	1.43	0.29	0.94	1.22	1.21	21.71
23:0	1.97	0.57	1.38	1.83	1.80	22.70
24:0	3.78	1.14	2.69	3.16	3.13	23.70
25:0	4.16	1.62	3.34	4.17	3.68	24.71
26:0	6.10	2.47	5.78	6.58	5.10	25.72
27:0	2.34	1.43	1.98	2.33	2.09	26.71
28:0	4.79	2.04	4.36	0.28	4.65	27.71
29:0	0.25	0.00	0.25	0.00	0.68	28.71
30:0	0.00	0.00	0.24	0.00	0.27	29.70

TABLE I (*continued*)

Composition (%)[a]	Linnaeus Terrace		Mount Fleming			
	A856-100[b]	A867-110[c]	A834-576[c]	A845-523[c]	A856-82f[c]	ECL[d]
Isoprenoid	10.73	1.45	2.14	5.44	19.28	
16:0	0.14	0.00	0.16	0.18	0.74	–
18:0	0.40	0.28	0.28	0.20	0.52	–
19:0	1.39	0.57	0.45	1.28	1.55	–
20:0	2.18	0.61	0.33	0.30	0.37	–
20:1a[e]	1.26	0.00	0.16	0.79	4.51	–
20:1b[e]	1.89	0.00	0.33	1.36	4.29	–
20:1c[e]	3.47	0.00	0.42	1.33	7.31	–
Content (μg/g of dry sample)	1.2	0.045	0.11	0.20	0.31	

[a] Carbon chain length:number of double bonds.
[b] Living community.
[c] Fossil community.
[d] Equivalent carbon chain length values of *anteiso*-alkanes for the DB-5 column.
[e] Structures were not determined.

TABLE II

FATTY ACID COMPOSITIONS AND CONTENTS FOR ROCK SAMPLES COLONIZED BY CRYPTOENDOLITHIC LICHEN-DOMINATED MICROBIAL COMMUNITIES, ANTARCTICA

Communities as defined in Table I.

Composition (%)[a]	Linnaeus Terrace		Mount Fleming			
	A856-100[b]	A867-110[c]	A834-576[c]	A845-523[c]	A856-82f[c]	ECL[d]
n-Alkanoic (short)	18.95	54.63	22.92	16.33	19.27	
9:0	1.04	0.32	0.83	0.49	0.53	–
10:0	0.28	0.31	0.28	0.26	0.17	–
11:0	0.09	0.15	0.10	0.08	0.04	–
12:0	0.37	2.34	0.30	0.30	0.16	–
13:0	0.09	0.36	0.13	0.14	0.05	–
14:0	0.67	5.87	3.09	1.02	0.74	–
15:0	0.32	3.03	0.98	0.53	0.41	–
16:0	9.66	24.65	13.28	8.09	10.48	–
17:0	0.34	1.75	0.30	0.50	0.39	–
18:0	5.29	14.67	3.19	4.16	5.36	–
19:0	0.81	1.19	0.45	0.78	0.94	–
n-Alkanoic (long)	21.55	34.68	22.29	34.93	35.78	
20:0	6.62	2.47	3.55	5.92	7.46	–
21:0	0.83	2.20	0.73	1.40	1.34	–
22:0	1.61	3.64	1.31	3.19	2.54	–
23:0	0.92	3.87	1.42	1.78	1.64	–
24:0	1.44	5.87	2.43	3.19	2.82	–
25:0	1.36	4.66	2.15	2.85	2.82	–
26:0	3.29	4.91	3.02	5.86	5.90	–
27:0	1.70	2.58	2.48	3.78	3.86	–
28:0	3.17	3.77	4.05	5.29	5.77	–
29:0	0.30	0.47	0.71	1.06	0.91	–
30:0	0.17	0.25	0.41	0.61	0.56	–
31:0	0.09	0.00	0.03	0.00	0.04	–
32:0	0.06	0.00	0.00	0.00	0.11	–

(Continued on p. 274)

TABLE II (*continued*)

Composition (%)[a]	Linnaeus Terrace		Mount Fleming			
	A856-100[b]	A867-110[c]	A834-576[c]	A845-523[c]	A856-82f[c]	ECL[d]
n-Alkenoic	44.49	2.88	40.23	27.94	25.25	
16:1	1.86	0.00	0.38	1.74	0.50	–
17:1	0.58	0.00	0.66	2.57	0.00	–
18:1	10.65	1.53	11.96	8.62	8.98	–
18:2	17.86	1.35	19.91	6.98	10.00	–
18:3	13.54	0.00	7.32	8.03	5.77	–
Iso	1.00	1.30	0.24	2.64	0.45	
12:0	0.00	0.00	0.00	0.11	0.00	11.63
13:0	Trace	0.00	0.00	0.02	0.00	12.63
14:0	0.12	0.46	0.06	0.42	0.05	13.63
15:0	0.58	0.28	0.05	1.12	0.19	14.63
16:0	0.30	0.45	0.13	0.88	0.21	15.64
17:0	0.00	0.12	0.00	0.09	0.00	16.63
Anteiso (short)	0.87	1.00	0.86	2.15	0.72	
13:0	Trace	0.09	0.00	0.05	0.00	12.73
15:0	0.46	0.54	0.41	1.31	0.37	14.73
17:0	0.24	0.36	0.11	0.65	0.18	16.74
19:0	0.16	0.00	0.34	0.15	0.17	18.74
Anteiso (long)	11.69	4.71	11.76	13.53	17.43	
20:0	0.18	0.00	0.12	0.20	0.20	19.74
21:0	0.10	0.12	0.07	0.11	0.16	20.73
22:0	0.18	0.14	0.90	0.23	0.23	21.74
23:0	0.37	0.24	0.30	0.40	0.51	22.73
24:0	0.81	0.24	0.57	0.72	1.00	23.74
25:0	2.35	0.51	1.57	2.17	2.84	24.74
26:0	1.56	0.55	2.01	1.97	2.80	25.75
27:0	4.37	1.28	3.65	4.71	5.86	26.75
28:0	0.53	0.59	0.78	0.86	1.09	27.75
29:0	1.25	1.06	1.72	2.08	2.67	28.75
30:0	0.00	0.00	0.06	0.10	0.08	29.74
Branched[e]	1.45	0.81	1.71	2.48	1.09	
15:0	0.10	0.21	0.11	0.19	0.11	–
16:0	0.14	0.00	0.17	0.25	0.17	–
17:0	0.75	0.24	0.41	1.01	0.26	–
18:0	0.11	0.36	0.11	0.43	0.11	–
19:0	0.36	0.00	0.91	0.50	0.43	–
Content (μg/g of dry sample)	79	0.31	5.4	14	58	

[a] Carbon chain length:number of double bonds.
[b] Living community.
[c] Fossil community.
[d] Equivalent carbon chain length values of *iso-* and *anteiso-* alkanoic acids for the DB-5 column.
[e] Structures were not determined.

ty acids were fairly low, less than 1.2 and 79 μg per gram of dry sample (Tables I and II), probably because of the low biomass in the harsh natural environment of the Ross Desert.

Sources of long-chain anteiso-alkanes and long-chain anteiso-alkanoic acids

Anteiso-alkanes are uncommon in the plant kingdom but are found in certain vascular plants. For instance, long-chain *anteiso*-alkanes having even-carbon numbers (a-C_{28} to a-C_{34}) are detected in various tobaccos with a predominance of a-C_{32} [21,22]. Even-carbon-numbered long-chain *anteiso*-alkanes (a-C_{24} to a-C_{32}) are also found in a number of insects [23,24]. They are usually accompanied by *n*- and other methyl-branched alkanes. Odd-carbon-numbered *anteiso*- alkanes (a-C_{15} to a-C_{29}) at a peak of a-C_{27} and odd-carbon-numbered *anteiso*-alkanoic acids (a-C_{13} to a-C_{31}) at a peak of a-C_{25} were detected in a wool wax by GC together with *n*- and *iso*-isomers of various compounds, including 2- and ω-hydroxy acids [25]. Vascular plants, insects and sheep do not occur in the sampling sites in the Ross Desert, and are unlikely sources of these *anteiso* compounds. No long-chain *anteiso*-alkanes and long-chain *anteiso*-alkanoic acids were detected in any lake sediment, pond sediment or soil sample in the Ross Desert or elsewhere in Antarctica, although long-chain alkanes and long-chain *anteiso*-alkanes are often major components in these samples [18,26–29]. These *anteiso* compounds were also not present in uncolonized Beacon Supergroup rock samples [30,31]. Hence long-chain *anteiso*-alkanes and long-chain *anteiso*-alkanoic acids are characteristic compounds of some cryptoendolithic microbial communities.

Cryptoendolithic micribial communities are fairly simple, composed of microalgae and cyanobacteria as primary producers, black and colorless fungi as consumers and heterotrophic bacteria as decomposers [2]. No secondary consumers are present as in the case of lakes in the McMurdo Dry Valleys [32]. Long-chain *anteiso*-alkanes and long-chain *anteiso*-alkanoic acids were found in the three microbial communities (lichen-dominated, red *Gloeocapsa* and *Hormathonema–Gloeocapsa* communities), but not in every sample (unpublished results). No long-chain *anteiso*-alkanes or long-chain *anteiso*-alkanoic acids were found in samples of granite or Koettlitz marble colonized by algae and lichens of the Ross Desert, and they were not detected in Nubian sandstone samples from the Negev Desert colonized by cryptoendolithic cyanobacteria (unpublished results).

These results can best be explained by microbial origin of long-chain *anteiso*-alkanoic acids and long-chain *anteiso*-alkanes, as they are characteristic of Antarctic cryptoendolithic microbial communities but not present in all samples. These organisms have adapted to a wide range of environments and to water and pH conditions (3.7–8.2) that vary widely [4], but analysis of organisms isolated from the rocks colonized by cryptoendolithic microbial communities (including five algae, four cyanobacteria, one lichen-forming fungus, three parasymbiotic black fungi, one yeast and sixteen bacteria) could not detect the presence of these compounds (unpublished results). *Anteiso*- and *iso*-alkenes ranging form a-C_{23} to a-C_{32} are found with the most predominant *anteiso*-alkanes of a-$C_{25:1}$, a-$C_{27:1}$ or a-$C_{29:1}$ in some bacteria [20,33,34]. Hence they may be derived from other organisms, *e.g.*, heterotrophic bateria that have not yet been isolated in culture [35]. The presence of short-chain *iso*- and *anteiso*-C_{12}–C_{17}-alkanoic acids (Table II), in addition to hopanoids ranging from C_{27} to C_{35} with a predominance of C_{30} (unpublished results), supports a considerable contribution of heterotrophic bacteria to these rock samples.

However, it is also possible that these unusual long-chain *anteiso* compounds arise from symbiotic processes rather than a single microorganism, as lichenic acids are only produced by lichens (symbiotic processes with green algae and fungi).

The coexistence of long-chain *anteiso*-alkanes and long-chain *anteiso*-alkanoic acids strongly suggests that long-chain *anteiso*-alkanes are derived from long-chain *anteiso*-alkanoic acids through microbial decarboxylation in the microbial communities [36]. The biosynthetic pathways of these *anteiso* compounds are not yet clear.

Long-chain *anteiso*-alkanoic acids and long-chain *anteiso*-alkanes are well preserved in rock samples with fossilized communities so they will probably be useful as biomarkers in environmental and geochemical studies. The long-chain *anteiso*-alkanoic acids may be important sources of long-chain *anteiso*-alkanes found in various geological environments. The long-chain *anteiso*-compounds also suggest the existence of a unique biosynthetic pathway in microorganisms. Further detailed studies using carbon isotopes (^{14}C or ^{13}C) are needed.

CONCLUSIONS

Hydrocarbons and fatty acids in rocks occupied by living and fossilized cryptoendolithic microbial

communities from the Ross Desert of Southern Victoria Land, Antarctica, were analyzed by capillary GC–MS. Long-chain *anteiso*-alkanes and long-chain *anteiso*-alkanoic acids were found in many samples, together with long-chain *n*-alkanes and *n*-alkanoic acids. These unusual compounds are probably derived from unidentified heterotrophic bacteria or formed through symbiotic processes in unique cryptoendolithic microbial communities. Long-chain *anteiso*-alkanes are probably derived from long-chain *anteiso*-alkanoic acids through microbial decarboxylation in the microbial communities. These *anteiso* compounds may be useful as new biomarkers in environmental and geochemical studies.

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