



# Novel brominated lipidic compounds from lichens of Central Asia

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## Abstract

The composition of novel brominated aliphatic compounds from lichens collected during summer from stones around the lake Issyk-Kul (Central Asia) is described. The compounds, predominantly fatty acid derivatives with unique groups (bromine, cyclopropane, oxirane) and conjugated double and triple bonds, were identified by means of <sup>1</sup>H and <sup>13</sup>C-NMR, MS, IR and UV spectra. © 1999 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Lichens are symbiotic organisms combining algal and fungal properties. Adaptability of such organisms to extreme environmental conditions, particularly to temperature-induced ones, is of much interest (Dembitsky, 1992). The Tian Shan mountain system in Central Asia has several lakes. Conspicuous among them is Issyk-Kul, a large and brackish mountain lake. The water of the lake has a fairly high salt content (up to 5.8 g/l). This content of salts, predominantly sulfates and chlorides, makes the water undrinkable. The flora around the lake is poor. Given the salty soil and extreme temperature differences between day and night, lichens are practically the only organisms to grow there.

In a previous paper (Řezanka, & Dembitsky, 1998) we have described the isolation of two halogenated acetylenic C<sub>18</sub> straight-chain fatty acids from terrestrial lichens of the genus *Acorospora gobiensis*. Brominated acetylenic acids show a variety of biological activities, e.g. antimicrobial, cytotoxic and ichthyotoxic; they are inhibitory for H, K-ATPase and HIV protease (Hirsh,

Carmely, & Kashman, 1987; Bourguet-Kondracki, Rakotoarisoa, Martin, & Guyot, 1992; Patil et al., 1992; Fusetani, Li, Tamura, & Matsunaga, 1993; Ichiba, Scheuer, & Kellyborges, 1994). In our continuing search for bioactive compounds from organisms of Central Asia we have isolated a brominated straight-chain acetylenic acid and we describe herein its structure elucidation by <sup>1</sup>H and <sup>13</sup>C-NMR spectra, MS, IR and UV spectra.

## 2. Results and discussion

The lichens *Cladonia furcata*, *Lecanora fructulosa*, *Leptogium saturninum*, *Parmelia linctina*, *P. comtseliadalis*, *Peltigera canina* and *Xanthoria* sp. were collected around the lake Issyk-Kul in Tian Shan mountains (Central Asia) in August 1995. The lichens were extracted three times with CHCl<sub>3</sub>–MeOH 1:1. Fractionation of this extract on Sephadex LH-20 column and reversed phase HPLC yielded eight compounds described as A<sub>1</sub>–H<sub>1</sub>, see Section 3 and Table 1. These compounds represented eight straight-chain, unsaturated, acetylenic brominated acids, which were, after methylation by diazomethane, identified on the basis of spectral data as: 16,18-dibromo-(15E,17Z)-octadeca-15,17-diene-5,7-diynoic acid methyl ester (C);

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Table 1  
Occurrence of brominated fatty acids from lichens of Tian Shan mountains

Name of lichen	Name of compounds (mg/50 g of dry) weight						
	A	B	C	D	E	F	G
<i>A. gobiensis</i>	13.1	8.8	— <sup>a</sup>	—	—	—	—
<i>C. furcata</i>	1.7	2.4	—	3.3	0.4	—	—
<i>L. fructulosa</i>	—	—	3.2	—	—	—	—
<i>L. saturninum</i>	0.4	4.2	—	1.9	—	—	5.1
<i>P. linctina</i>	—	—	6.3	—	2.8	—	—
<i>P. comteliadalis</i>	2.2	2.5	0.3	5.8	—	3.0	—
<i>P. canina</i>	0.9	—	—	0.3	—	9.4	4.8
<i>Xanthoria</i> sp.	—	0.8	2.4	—	5.7	—	—

<sup>a</sup> Less than 0.1 mg/50 g of dry weight.

18,18-dibromo-17-octadecene-5,7-diynoic acid methyl ester (D); 18-bromo-(5E,17Z)-octadeca-5,7-diene-15-ynoic acid methyl ester (E); 6-bromo-(5E,15Z)-octadeca-5,15-diene-11,13,17-triynoic acid methyl ester (F); 18-bromo-9-hydroxy-12,13-trans-epoxy-(10E,15Z)-octadeca-10,15-diene-17-ynoic acid methyl ester (G); 18-bromo-5,6-trans-endomethylene-7,11,15-trimethyl-(8E,10Z)-octadeca-8,10-diene-17-ynoic acid methyl ester (H).

A and B were reported earlier to occur in the terrestrial lichen *A. gobiensis* collected also in Tian Shan mountains; they were characterized by compari-

son of spectral data with literature (Řezanka, & Dembitsky, 1999) and Table 2 or Table 3 and Section 3.

Compound C was a colorless oil, giving a pseudo-molecular ions at  $m/z$  460, 464 and 466 ( $M + NH_4$ )<sup>+</sup> in its CI-MS. The IR spectrum indicated an ester (1730  $cm^{-1}$ ) and disubstituted acetylenic group (2223  $cm^{-1}$ ). Also significant was the absorbance at 980  $cm^{-1}$  (E configuration of double bond) of this ester. The <sup>13</sup>C-NMR spectrum Table 3 contained signals indicative of a bis-acetylenic group ( $\delta$  from 61.2 to 79.1 ppm), four methine olefinic carbons and carbonyl, which accounted for the overall of unsaturation. The <sup>1</sup>H-NMR spectrum of C displayed three olefinic protons at 6.07, 6.38 and 6.66 ppm while the allylic and other methylene groups occur at  $\delta$  1.5–2.4 ppm. Characterization of C was based on extensive decoupling experiments and <sup>1</sup>H-<sup>1</sup>H 2D COSY spectra. A COSY experiment revealed allylic coupling ( $J=1.1$  Hz) between H15 and H17 in addition to the sequential vicinal and very small homoallylic correlation from H14 (br q) to H17 (br dd). The three olefinic doublets at  $\delta$  6.38 ppm ( $J=7.7$  Hz) and a signal at  $\delta=6.66$  ppm (dd  $J=7.7$ ; 1.1 Hz) indicated a Z disubstituted double bond. The last olefinic hydrogen  $\delta=6.07$  ppm was dt ( $J=7.6$ ; 1.1 Hz) corresponding to a hydrogen on a trisubstituted double bond joined on the monosubstituted side to a CH<sub>2</sub> group. Irradiation in the allylic region simplified dt at 6.07 ppm to dd

Table 2  
<sup>13</sup>C-NMR spectra of brominated fatty acids from Tian-Shan lichens

Carbon No.	Structure of brominated fatty acids							
	A	B	C	D	E	F	G	H
1	179.1 s	178.8 s	179.4 s	179.2 s	179.2 s	177.6 s	175.7 s	179.3 s
2	33.9 t	33.1 t	32.7 t	32.9 t	33.8 t	33.9 t	34.9 t	34.2 t
3	24.2 t	23.3 t	23.6 t	23.4 t	24.2 t	25.1 t	24.6 t	24.2 t
4	32.4 t	18.5 t	18.7 t	18.6 t	32.3 t	32.6 t	28.7–29.2 t	34.6 t
5	130.8 d	79.7 s	79.1 s	79.6 s	130.7 d	131.9 d	28.7–29.2 t	17.8 d
6	129.5 d	63.1 s	61.2 s	62.4 s	129.6 d	124.8 s	28.7–29.2 t	18.8 d
7	32.4 t	68.5 s	65.2 s	72.8 s	32.5 t	32.1 t	28.7–29.2 t	30.9 d
8	29.6 t	78.9 s	77.7 s	80.1 s	29.7 t	29.3 t	30.0 t	124.8 d
9	29.2 t	19.0 t	19.1 t	19.2 t	29.3 t	28.6 t	75.7 d	135.9 d
10	29.5 t	27.9 t	28.1 t	28.2 t	29.5 t	19.8 t	134.5 d	133.3 d
11	29.3 t	28.7 t	28.4 t	28.5 t	29.3 t	66.4 s	129.9 d	136.8 s
12	28.8 t	28.8 t	28.6 t	28.5 t	28.8 t	72.3 s	58.1 d	39.6 t
13	28.9 t	28.9 t	30.9 t	28.3 t	28.9 t	78.5 s	61.0 d	25.2 t
14	19.1 t	28.6 t	32.6 t	28.5 t	19.2 t	83.9 s	29.9 t	36.4 t
15	92.7 s	27.6 t	109.9 d	27.4 t	92.6 s	111.7 d	119.7 d	28.6 d
16	77.5 s	18.5 t	115.4 s	32.2 t	77.1 s	109.9 d	118.1 d	18.5 t
17	118.0 d	83.2 s	136.5 d	139.2 d	115.7 d	82.6 s	92.8 s	83.2 s
18	117.1 d	84.5 s	131.0 d	88.1 s	116.8 d	79.4 d	85.4 s	84.5 s
19								11.7 t
20								19.7 q
21								25.6 q
22								19.5 q
OMe	51.3 q	51.3 q	51.2 q	51.2 q	51.2 q	51.7 q	51.4 q	51.5 q

Table 3  
<sup>1</sup>H-NMR spectra of brominated fatty acids from Tian-Shan lichens

H on C <sub>x</sub>	Structure of brominated fatty acids	A	B	C	D	E	F	G	H
1	–	–	–	–	–	–	–	–	–
2	2.32 t (2H, <i>J</i> = 7.5 Hz)	2.39 t (2H, <i>J</i> = 7.3 Hz)	2.39 t (2H, <i>J</i> = 7.4 Hz)	2.38 t (2H, <i>J</i> = 7.5 Hz)	2.32 t (2H, <i>J</i> = 7.5 Hz)	2.29 t (2H; 7.4)	2.39 t (2H)	2.39 t (2H)	2.42 t (2H)
3	1.63 quin (2H, <i>J</i> = 7.5 Hz)	1.88 tt (2H, <i>J</i> = 7.3; 6.7 Hz)	1.87 tt (2H, <i>J</i> = 7.4; 6.9 Hz)	1.86 tt (2H, <i>J</i> = 7.5; 6.8 Hz)	1.63 quin (2H, <i>J</i> = 7.5 Hz)	1.63 m (2H)	1.63 m (2H)	1.63 m (2H)	1.63 m (2H)
4	1.97 m (2H)	2.33 tt (2H, <i>J</i> = 6.7; 0.8 Hz)	2.31 tt (2H, <i>J</i> = 6.9; 0.8 Hz)	2.32 tt (2H, <i>J</i> = 6.8; 0.8 Hz)	1.97 m (2H)	2.13 q (2H; 7.5)	1.35–1.42 m (2H)	1.35–1.42 m (2H)	1.21 m (2H)
5	5.38 dt (1H, <i>J</i> = 15.5; 7.4 Hz)	–	–	–	5.38 dt (1H, <i>J</i> = 15.5; 7.4 Hz)	5.91 t (2H; 7.7)	1.35–1.42 m (2H)	1.35–1.42 m (2H)	0.42 m (1H)
6	5.34 dt (1H, <i>J</i> = 15.5; 7.0 Hz)	–	–	–	5.34 dt (1H, <i>J</i> = 15.5; 7.0 Hz)	–	1.35–1.42 m (2H)	1.35–1.42 m (2H)	0.42 m (1H)
7	1.95 m (2H)	–	–	–	1.95 m (2H)	2.63 m (2H)	1.35–1.42 m (2H)	1.35–1.42 m (2H)	2.14 dd (1H; 15.0; 7.1)
8	1.53 m (2H)	–	–	–	1.53 m (2H)	1.45 m (2H)	2.05 ddt (2H; 16.3; 8.9; 3.6)	2.05 ddt (2H; 16.3; 8.9; 3.6)	5.78 dd (1H; 15.0; 14.5)
9	1.47 m (2H)	2.21 tt (2H, <i>J</i> = 6.9; 0.8 Hz)	2.22 tt (2H, <i>J</i> = 6.9; 0.8 Hz)	2.20 tt (2H, <i>J</i> = 6.9; 0.8 Hz)	1.47 m (2H)	1.39 m (2H)	4.38 tdd (1H; 5.7; 3.6; 1.4)	4.38 tdd (1H; 5.7; 3.6; 1.4)	6.59 dd (1H; 15.5; 14.5)
10	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	2.31 td (2H; 7.4; 0.6 <sup>a</sup> )	6.03 dd (1H; 15.6; 5.7)	6.03 dd (1H; 15.6; 5.7)	5.83 d (1H; 15.5)
11	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	–	5.57 ddd (1H; 15.6; 8.1; 1.4)	5.57 ddd (1H; 15.6; 8.1; 1.4)	–
12	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	1.47 m (2H)	–	3.24 dd (1H; 8.1; 1.9)	3.24 dd (1H; 8.1; 1.9)	2.03 m (2H)
13	1.47 m (2H)	1.45 m (2H)	1.40 br m (2H)	1.46 m (2H)	1.47 m (2H)	–	2.93 dt (1H; 5.7; 1.9)	2.93 dt (1H; 5.7; 1.9)	1.60 m (2H)
14	2.26 ddt (2H, <i>J</i> = 6.8; 2.3 <sup>a</sup> ; 0.6 <sup>b</sup> Hz)	1.47 m (2H)	2.06 q (2H, <i>J</i> = 6.6 Hz)	1.47 m (2H)	2.26 ddt (2H, <i>J</i> = 6.8; 2.0 <sup>a</sup> ; 0.6 <sup>b</sup> Hz)	–	2.37 br s (2H)	2.37 br s (2H)	1.35 m (2H)
15	–	1.61 m (2H)	6.07 dt (1H, <i>J</i> = 7.6; 1.1 Hz)	1.48 m (2H)	–	6.00 dd (1H; 10.8; 0.6 <sup>d</sup> )	6.44 dt (1H; 10.1; 6.9)	6.44 dt (1H; 10.1; 6.9)	2.08 dddd (1H; 10.9; 7.9; 5.7; 1.9) <sup>e</sup>
16	–	2.37 m (2H)	–	2.19 q (2H, <i>J</i> = 7.4 Hz)	–	5.43 dd (1H; 10.8; 1.1 <sup>e</sup> )	6.84 d (1H; 10.1)	6.84 d (1H; 10.1)	2.37 dd (1H; 17.0; 5.7) 2.49 dd (1H; 17.0; 7.9)
17	6.17 dt (1H, <i>J</i> = 14.0; 2.2 <sup>a</sup> Hz)	–	6.66 br dd (1H, <i>J</i> = 7.7; 1.1 Hz)	6.36 t (1H, <i>J</i> = 7.4 Hz)	6.38 dd (1H, <i>J</i> = 7.5; 2.0 <sup>b</sup> Hz)	–	–	–	–
18	6.57 d (1H, 14.0; 0.6 <sup>b</sup> Hz)	–	6.38 d (1H, <i>J</i> = 7.7 Hz)	–	6.54 dd (1H, <i>J</i> = 14.0; 0.6 <sup>b</sup> Hz)	3.07 d (1H; 1.1 <sup>c</sup> )	–	–	–
19	–	–	–	–	–	–	–	–	0.19 m (2H)
20	–	–	–	–	–	–	–	–	0.87 d (3H; 6.7)
21	–	–	–	–	–	–	–	–	1.72 s (3H)
22	–	–	–	–	–	–	–	–	0.85 d (3H; 7.0)
OMe	3.67 s (3H)	3.65 (3H)	3.66 s (3H)	3.68 s (3H)	3.65 s (3H)	3.64 s (3H)	3.65 s (3H)	3.65 s (3H)	3.65 s (3H)

<sup>a</sup> <sup>5</sup>*J*<sub>HH</sub>.

<sup>b</sup> <sup>6</sup>*J*<sub>HH</sub>.

<sup>c</sup> *J*<sub>15,16</sub> = 7.9; *J*<sub>15,16'</sub> = 5.7; *J*<sub>15,14</sub> = 10.9; *J*<sub>15,14'</sub> = 1.9 Hz.

<sup>d</sup> <sup>7</sup>*J*<sub>HH</sub>.

<sup>e</sup> <sup>4</sup>*J*<sub>HH</sub>.

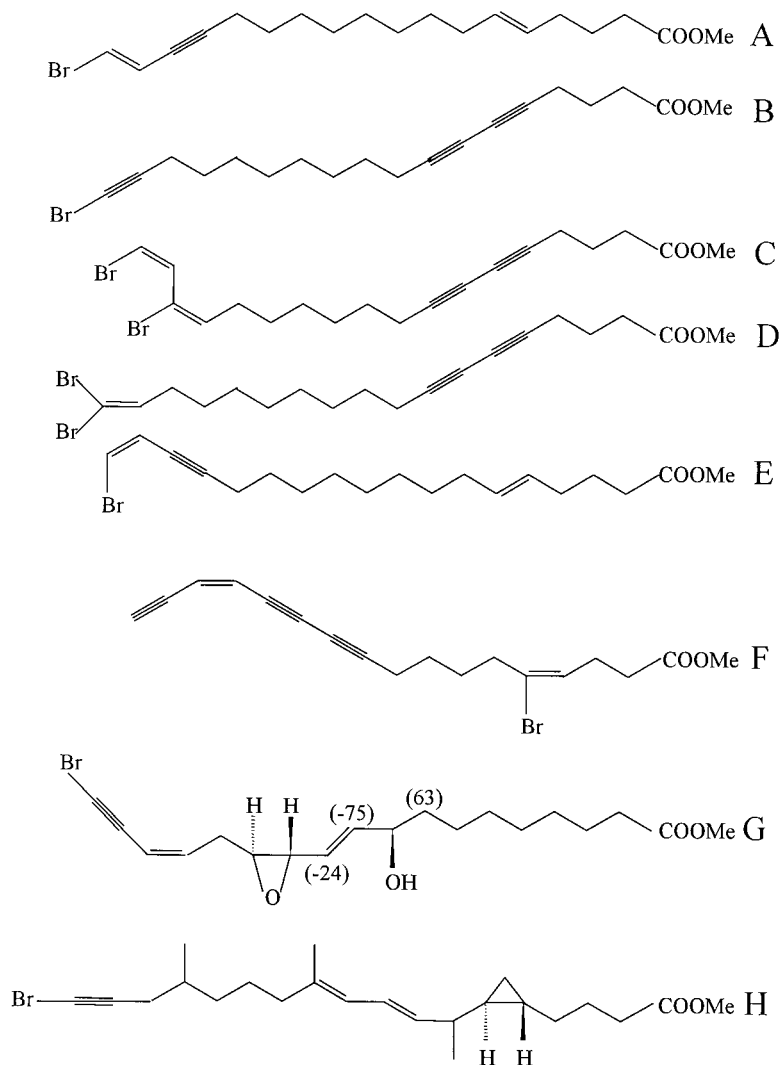


Fig. 1. Structure of novel brominated fatty acids from terrestrial lichens of Tian Shan mountains identified by means of  $^1\text{H}$  and  $^{13}\text{C}$ -NMR, MS, IR and UV spectra, see Section 3.

( $J_{15,17}=1.3$ ;  $J_{15,18}=0.7$  Hz respectively) and removed the small splitting ( $J=1.1$  Hz) from the 6.66 ppm signal.

The methyl ester of a fourth fatty acid compound (D) possesses the typical diyne structure in a UV spectrum. The  $^1\text{H}$ -NMR data, (see Table 3) confirmed the structure of D. COSY experiment assigned all the protons of the molecule.  $^{13}\text{C}$ -NMR spectrum ( $\delta$  139.2 d,  $\text{C}_{17}$ ; 88.1 s  $\text{C}_{18}$ ) indicated only one double bond. The high field  $^{13}\text{C}$ -NMR shift for  $\text{C}_{18}$  is due to a heavy atom effect caused by substitution with two bromine atoms. The other signals at lower field i.e. four signals from 60 to 80 ppm, revealed a diyne system. The spectra are very similar to the spectra of compounds formerly described (Hirsh et al., 1987), the only difference being the loss of additional double bonds. Our acid is more saturated than the compounds formerly described.

The acid designed as E is identical with compound

A described previously (Řezanka, & Dembitsky, 1999). The only difference between compounds A and E was in the configuration of the bromovinyl terminus, which was 17 E in A and 17 Z in E ( $J_{17,18}=14.0$  and 7.5 Hz, respectively).

The on-line recorded UV spectrum suggested that compound F was an entriyne system, i.e. had three triple bonds and only one double bond (the second double bond is not conjugated) at 210, 239, 252, 266 and 280 nm. The IR spectrum exhibited absorption bands characteristic of an ester ( $1730\text{ cm}^{-1}$ ) as well as absorption bands at  $2239\text{ cm}^{-1}$  and a weaker band at  $2251\text{ cm}^{-1}$ . The elemental composition of  $\text{C}_{19}\text{H}_{21}\text{O}_2\text{Br}$  was deduced from HRCIMS for compounds F (found  $m/z$   $[\text{M} + \text{NH}_4]^+$  379.3168, calcd 379.3171).  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectroscopy confirmed that compound F consisted of aliphatic spin systems separated by a single double bond and by a conjugated arrangement of three triple bonds and a double bond. Coupling

constants of olefinic protons indicated the configuration of F to be Z ( $J=10.8$  Hz). The calculated chemical shifts of H5 for both the E isomer ( $\delta$  5.36 ppm) and the Z isomer ( $\delta$  5.98 ppm) are more consistent with 5.62 configuration for E (5.91 t,  $J=7.7$  Hz) as shown in (Pascual, Meier, & Simon, 1966). The double bond geometry in F (5,6-double bond) was also confirmed by NOE difference spectroscopy. A Z geometry for C<sub>5</sub>–C<sub>6</sub> was indicated by observing enhancement of H5 when two H4 allylic methylene protons were irradiated.

The mass spectrum of G gave pseudomolecular ions at  $m/z$  416 and 418 and the molecular formula of  $[C_{19}H_{27}O_4Br + NH_4]^+$  was established by HRCIMS. The UV spectrum showed only end absorption, while in IR spectrum the bands belonged to double and triple bonds. Ester and also oxirane ring were observed, see Section 3.  $^{13}C$ -NMR spectrum indicated one ester carbon, four olefinic methine carbons, two carbons at 85.4 and 92.8 ppm (triple bond), a downfield oxygen-bearing methine (hydroxy group on C<sub>9</sub>), two relatively upfield oxygen-bearing methines assigned to an epoxide and nine methylenes. The epoxide carbons at 58.1 and 61.0 ppm were correlated to the protons at 3.24 and 2.93 ppm, respectively, by a HMQC experiment supporting the presence of a three-membered oxygen containing ring. The substitution of the side chain was established by considering the COSY and HMBC spectra and extensive decoupling experiments at 500 MHz. The E stereochemistry of the C<sub>10</sub>–C<sub>11</sub> double bond was assigned by the large 15.6 Hz vicinal coupling. The trans epoxide stereochemistry was deduced from small 1.9 Hz C<sub>12</sub>–C<sub>13</sub> vicinal coupling. The configuration of the hydroxy group was established by the modified Mosher method (Ohtani, Kusakmi, Kashman, & Kakisawa, 1991; Rieser et al., 1992). Both the *R* and *S* esters of G were produced from the *R*- and *S*-MTPA acid chlorides, respectively. After chromatographic cleanup, the  $^1H$ – $^1H$ -COSY experiments were used to assign the  $^1H$ -NMR spectra for each derivative. The difference (due to anisotropic effects) in chemical shifts between each set of like protons was tabulated using the equation  $\Delta\delta = \delta_S - \delta_R$ , see Fig. 1. The  $\Delta\delta$  values for MTPA ester of G appear to indicate that C<sub>9</sub> is *R*.

The CIMS of H displayed equal intensity  $[M]^+$  and  $[M+2]^+$  ions at  $m/z$  438 and  $m/z$  440, respectively, indicating that H contained one bromine atom. This fact was confirmed by HRCIMS measurement ( $m/z$  at 439.4567 C<sub>23</sub>H<sub>33</sub>BrO<sub>2</sub>). This molecular composition required H to have six double bond equivalents, one of which was due to ester carbonyl ( $^{13}C$ -NMR,  $\delta$  179.3 s; IR  $\nu_{C=O}$  1732 cm<sup>-1</sup>). Furthermore, four sp<sup>2</sup> hybridized carbons at  $\delta$  124.8, 133.3, 135.9 and 136.8 ppm indicated the presence of two double bonds. The chemical shifts at 83.2 and 84.5 ppm, both singlets, are proof of one triple bond substituted by bromine (loss

of signal of hydrogen), and the compound G contain a ring. Interpretation of  $^1H$ – $^1H$  and  $^1H$ – $^{13}C$  COSY data generated the structure of G, which was composed of a fatty acid ester with a 1,2-disubstituted cyclopropane ring between C<sub>4</sub> and C<sub>7</sub>. Characteristically shielded methylene protons (two H19) and two methine protons (H5, H6) (see Table 3) were also diagnostic for a cyclopropyl ring. An E geometry for H8 and H9 double bond was suggested to be establishing a 14.5 Hz coupling constant between these protons. Similarly, an E geometry for C<sub>10</sub>–C<sub>11</sub> was indicated by observing an enhancement of H<sub>10</sub> when H<sub>9</sub> allylic methine proton was irradiated. The substitution of a straight chain by three methyls was confirmed by HMBC experiment. Also, the splitting of the ester in mass spectrometer produced ions at  $m/z$  281 and 283, 235 and 305 or 145 and 147. This endorsed the structure of a phytanic acid homologue (isoprenoid acid).

Acetylenic acids are well-known constituents of higher terrestrial plants but to our knowledge only one study (Řezanka, & Dembitsky, 1999) described these acids from lower plants. Although the occurrence of bromine is common in marine natural products (Gribble, 1996) the number of brominated acids reported to date is still small and they are not present in lower or higher terrestrial plants.

### 3. Experimental

The total lipid extract from *A. gobiensis*, *C. furcata*, *L. fructulosa*, *L. saturninum*, *P. linctina*, *P. comtseliadalis*, *P. canina* and *Xanthoria* sp. (Řezanka, & Dembitsky, 1999), a viscous dark oil, was eluted from Sephadex LH-20 column with chloroform–hexane 65:35 and then separated by RP-HPLC (Supelco, RP-18, 85% MeOH–H<sub>2</sub>O). This afforded compounds A<sub>1</sub>–H<sub>1</sub> (3.2–13.8 mg from ca. 50 g of lichen). The free acids were treated with a solution of CH<sub>2</sub>N<sub>2</sub> in diethyl-ether. The crude methyl esters were further purified by RP-HPLC (Supelco, RP-18, 95% MeOH–H<sub>2</sub>O).

UV spectra were measured by the Cary 118 (Varian) apparatus in heptane within the range of 200–350 nm. A Perkin–Elmer Model 1310 (Perkin–Elmer, Norwalk, CT, USA) infrared spectrophotometer was used for scanning infrared spectroscopy of methyl esters as neat film. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz ( $^1H$ ), 125.7 MHz ( $^{13}C$ ). High- and also low-resolution mass spectra were recorded using a VG 7070E-HF spectrometer (positive chemical ionization mode with NH<sub>3</sub>, source temperature 130°C). Ionizing condition for CI was 70 eV.

18-bromo-(5E,17E)-octadeca-5,17-diene-15-ynoic acid methyl ester (A) UV  $\lambda_{max}$  (MeOH, nm) 214 ( $\epsilon$ 19800), 233 ( $\epsilon$ 11400); IR (as neat film) 2810, 2210,

1730, 1635, 1560, 1440 and 915  $\text{cm}^{-1}$ ; MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 386 and 388; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{29}\text{O}_2\text{Br} + \text{NH}_4$ )<sup>+</sup> 386.4771 and 388.4771, found 386.4769 and 386.4773 (intensity ratio 1:0.97).

18-bromooctadeca-5,7,17-triynoic acid methyl ester (B) UV  $\lambda_{\text{max}}$  (MeOH, nm) 225 ( $\epsilon$ 15800); IR (as neat film) 2221, 1740, 1608 and 1445  $\text{cm}^{-1}$ ; MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 382 and 384; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{25}\text{O}_2\text{Br} + \text{NH}_4$ )<sup>+</sup> 382.4461 and 384.4461, found 382.4457 and 384.4453 (intensity ratio 1:0.98).

16,18-dibromo-(15E,17Z)-octadeca-15,17-diene-5,7-diynoic acid methyl ester (C) UV  $\lambda_{\text{max}}$  (MeOH, nm) 205 ( $\epsilon$ 17000) 212 ( $\epsilon$ 16200) 225 ( $\epsilon$ 18000) 250 ( $\epsilon$ 6200) and 264 ( $\epsilon$ 4200); IR (as neat film) 3010, 2930, 2223, 1741, 1590 and 980  $\text{cm}^{-1}$ ; MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 460 and 462 and 464; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{24}\text{O}_2\text{Br}_2 + \text{NH}_4$ )<sup>+</sup> 460.4372, 462.4371 and 464.4371, found 460.4374, 462.4372 and 464.4372 (intensity ratio 2:1.97:1.94).

18,18-dibromo-17-octadecene-5,7-diynoic acid methyl ester (D) UV  $\lambda_{\text{max}}$  (MeOH, nm) 215 ( $\epsilon$ 13200) 231 ( $\epsilon$ 4800) and 249 ( $\epsilon$ 1800); IR (as neat film) 3005, 2941, 2222, 1735, and 1575  $\text{cm}^{-1}$ . MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 462 and 464 and 466; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{26}\text{O}_2\text{Br}_2 + \text{NH}_4$ )<sup>+</sup> 462.4533, 464.4533 and 466.4534, found 462.4532, 464.4530 and 466.4529 (intensity ratio 2:1.96:1.92).

18-bromo-(5E,17Z)-octadeca-5,7-diene-15-ynoic acid methyl ester (E) UV  $\lambda_{\text{max}}$  (MeOH, nm) 231 ( $\epsilon$ 10500); IR (as neat film) 2815, 2220, 1730, 1631, 1560 and 1440  $\text{cm}^{-1}$ . MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 386 and 388; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{29}\text{O}_2\text{Br}_2 + \text{NH}_4$ )<sup>+</sup> 386.4771 and 388.4770, found 386.4769 and 388.4768 (intensity ratio 1:0.97).

6-bromo-(5E,15Z)-octadeca-5,15-diene-11,13,17-triynoic acid methyl ester (F) UV  $\lambda_{\text{max}}$  (MeOH, nm) 214 ( $\epsilon$ 7500), 229 ( $\epsilon$ 9100), 240 ( $\epsilon$ 8650), 253 ( $\epsilon$ 13500), 268 ( $\epsilon$ 23000) and 284 ( $\epsilon$ 16800); IR (as neat film) 3300, 2955, 2236, 2120, 1730, 0000 and 000  $\text{cm}^{-1}$ . MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 378 and 380; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{21}\text{O}_2\text{Br} + \text{NH}_4$ )<sup>+</sup> 378.4131

and 380.4130, found 378.4129 and 380.4127 (intensity ratio 1:0.97).

18-bromo-9-hydroxy-12,13-trans-epoxy-(10E,15Z)-octadeca-10,15-diene-17-ynoic acid methyl ester (G) UV  $\lambda_{\text{max}}$  (MeOH, nm) 232 ( $\epsilon$ 8400); IR (as neat film) 2820, 2225, 1732, 1280, 890 and 830  $\text{cm}^{-1}$ . MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 416 and 418; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{19}\text{H}_{27}\text{O}_4\text{Br} + \text{NH}_4$ )<sup>+</sup> 416.4592 and 418.4591, found 416.4589 and 418.4588 (intensity ratio 1:0.97).

18-bromo-5,6-trans-endomethylene-7,11,15-trimethyl-(8E,10Z)-octadeca-8,10-diene-17-ynoic acid methyl ester (H) UV  $\lambda_{\text{max}}$  (MeOH, nm) 236 ( $\epsilon$ 29000); IR (as neat film) 2960, 2227, 1738, 1608, 1305, 1015, 984, 857 and 835  $\text{cm}^{-1}$ . MS–CI ( $\text{NH}_3$ ,  $m/z$ ) ( $\text{M} + \text{NH}_4$ )<sup>+</sup> 438 and 440; HR–MS–PCI ( $\text{NH}_3$ ,  $m/z$ ) calcd for ( $\text{C}_{23}\text{H}_{33}\text{O}_2\text{Br} + \text{NH}_4$ )<sup>+</sup> 438.5523 and 440.5521, found 438.5522 and 440.5520 (intensity ratio 1:0.97).

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