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Bromoallenic lipid compounds from lichens of central Asia

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Abstract

The composition of two bromoallenic aliphatic fatty acids obtained from lichens collected around Lake Issyk-Kul (central Asia) is described. These compounds were identified by means of ^{1}H and ^{13}C NMR, MS and IR spectra. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Bromoallenic acid; Lichens; *Acarospora gobiensis*

1. Introduction

Although the lipid composition of lichen species is well documented (Dembitsky, 1992), there is only a limited amount of information on the lipids and fatty acids present in lichens growing in the Tian Shan Mountains.

The presence of various chemical components in lichens, in particular aromatic compounds, carotenoids (Huneck and Yoshimura, 1996) and fatty acids (Dembitsky, 1992) has long been used for chemotaxonomic purposes. The fatty acid composition of lichens suggests a great biochemical diversity of these organisms (Dembitsky et al., 1991, 1992; Řezanka and Dembitsky, 1998, 1999a). The present work reports on the ^{1}H and ^{13}C NMR, MS and IR spectroscopic analysis of Tian Shan lichen fatty acids which revealed unusual bromoallenic fatty acids.

2. Results and discussion

In connection with our phytochemical interest in lichens, we examined specimens of *Acarospora gobiensis* H. Magn.; *Cladonia furcata* (Huds.) Schrad., *Lecanora fructulosa* (Dicks.) Ach.; *Leptogium saturninum* (Dicks.) Nyl.; *Peltigera canina* (L.) Willd.; *Rhizoplaca peltata* Ram.; *Xanthoparmelia camtschadalis* (Ach.) Hale, *Xanthoparmelia tinctina* (Maheu & Gill.) Hale and *Xanthoria elegans* (Link.) Th. Fr. collected in August 1995 around the Lake Issyk-Kul in the Central Asian Tian Shan Mountains, and which proved to be a rich source of halogenated metabolites (Řezanka and Dembitsky, 1998, 1999a; Řezanka and Guschina, 1999).

The new bromoallenes (**1** and **2**) as well as some known compounds (Řezanka and Dembitsky, 1999b) and bromine-acetylenic fatty acids (Řezanka and Dembitsky, 1998, 1999a), were isolated from the chloroform-soluble part of the extract of these lichens by a combination of column chromatography on Sephadex LH-20 and preparative HPLC.

Compound **1**, mp 129°, $[\alpha]_D$ +142 showed the presence of a hydroxyl group (3400 cm^{-1}), an allene (1958 cm^{-1}) and a carboxyl group (1710 cm^{-1}) in its IR spectrum. The acid **1** had a molecular formula $C_{18}H_{29}BrO_3$, which was established by HR–MS–PCI (high-resolution mass spectrometry with positive chemical ionization). The mass spectrum from low-resolution PCI-MS contained peaks at m/z 358, 360 $[M+NH_4]^+$ and peak at m/z 234 derived from $[M+NH_4]^+$ by successive losses of C_3H_2Br. The ^{1}H and ^{13}C-NMR spectra indicated the existence of a bromoallene side chain [δ 5.51 (1H, *dd*, $J=5.5$, 6.0 Hz) and 6.09 (1H, *dd*, $J=5.5$, 1.5 Hz); δ 201.9, 102.4 and 74.1] and a double bond [δ 5.39 (1H, *ddd*, $J=9.7$, 7.1 and 6.2 Hz) and 5.46 (1H, *ddd*, $J=9.7$, 5.7 and 0.6 Hz); δ 127.0 and 132.1]. Furthermore, the *Z*-configuration of the double bond at C-12 was indicated by the values ($J=9.7$ Hz) of the coupling constants between the pertinent olefinic protons. Further a downfield methane carbon, i.e. attributed to C_{15} which bears a hydroxy group and eleven methylenes belonging to a long chain were indicated in the ^{13}C NMR spectrum (Table 2). The

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configuration of the hydroxyl group was established by two independent methods. One of them is the modified method of Mosher (Ohtani et al., 1991; Rieser et al., 1992), in which the reaction of the hydroxyl group with *R*- and *S*-MTPA acid chlorides gives *S* and *R* esters. The configuration of these esters was determined by calculating the differences in the chemical shifts of the appropriate hydrogens caused by anisotropic effects. The results given in Fig. 1 show that C_{15} has an S configuration.

Detailed ^1H NMR spin decoupling experiments on **1** combined with a 2D COSY (^1H–^1H) spectrum of **1** led to the following structure:

$$HOOC-(CH_2)_{10}-CH=CH-CH_2-CHX-CH=C=CHX \text{ where } X=O \text{ or } Br$$

The full confirmation of structure **1** (and also **2**) was obtained by the second method, which involved hydrogenation of **1** over platinum oxide in ethanol to give the fully saturated compound **1a**, $C_{18}H_{36}O_3$. The mass spectrum of **1a**, like that of 15-hydroxyoctadecanoic acid, revealed a prominent peak at *m*/*z* 257, ascribable to a fragment caused by elimination of a C_3 unit, and a base peak at *m*/*z* 225 (loss of MeOH from peak at *m*/*z* 257).

Further, the position of a hydroxyl group was established by fragmentation of the TMS ether of the hydroxy pyrrolidide as shown in Fig. 2; intensities of the appropriate ions are listed in the Experimental. The TMS ether has two nearly equivalent centers where ionization and fragmentation can occur. Fragmentation gives the ion **D**, which contains the terminal methyl part of the chain and is formed by α-cleavage on the amide side chain of the oxygenated carbon with the charge remaining on the silyl oxygen. The ion **D** is intense in the spectrum of 15-trimethylsilyloxy pyrrolidide, indicating that fragmentation occurs more readily when the oxygenated carbon is well separated from the amide group. The second ion **C** results from the transfer of the TMS group to the amide carbonyl followed by a second α-cleavage on the amide side chain of the oxygenated carbon. Ion **C** is rather weak (3%) in the spectra listed in Experimental. Ion **B** was expected in the spectrum of the TMS derivative because hydrogen migration is likely. Ion **A**, the other expected ion, results from α-cleavage on the methyl terminal side of the oxygenated carbon. This ion is also strong. Another important ion is the $[M\text{-}15]^+$ ion, usually prominent in the spectra of silyl ethers (Tulloch, 1985), formed by loss of a methyl group. The intensity is about 20%. The $[M\text{-}90]^+$ ion (loss of TMSiOH) and M^+ are weak ions in the spectrum.

Chiral HPLC was used to confirm the chirality of the carbon bearing the hydroxyl group. The enantiomers of both hydroxy fatty acids **1a** and **2a** (i.e. fully hydrogenated **1** and **2**) were separated by HPLC using a chiral stationary phase. In this paper, we demonstrate that chiral HPLC on a (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-phenylglycine (DNBPG) column provides a convenient methods for analyzing the steric configurations of 15-hydroxystearic acid.

The methyl esters of **1a** and/or **2a** were reacted with naphthoyl chloride in pyridine (the *R* or *S* configuration of the chiral center is not changed by derivatization) and the resulting aromatic esters were separated by chiral HPLC. The enantiomers of the hydroxy fatty acid methyl naphthoyl esters separated with good resolution (the α value is 1.08). For methyl esters derived by naphthoylation of a secondary alcohol group, the *R* enantiomer elutes more rapidly than the *S* enantiomer. This is in accordance with the literature reports (Hawkins et al., 1988).

In view of the strong positive rotation of **1**, the bromoallene moiety of **1** was assigned the *S*-configuration

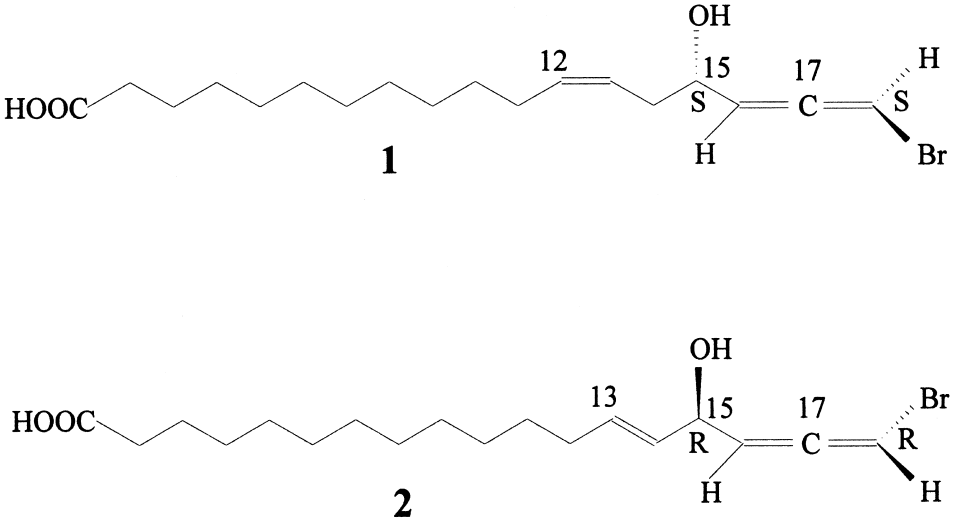


Fig. 1. The structures of the (12*E*, 15*S*, 18*S*)-15-hydroxy-18-bromo-12,16,17-octadecatrienoic acid (**1**) and (13*Z*, 15*R*, 18*R*)-15-hydroxy-18-bromo-13,16,17-octadecatrienoic acid (**2**).

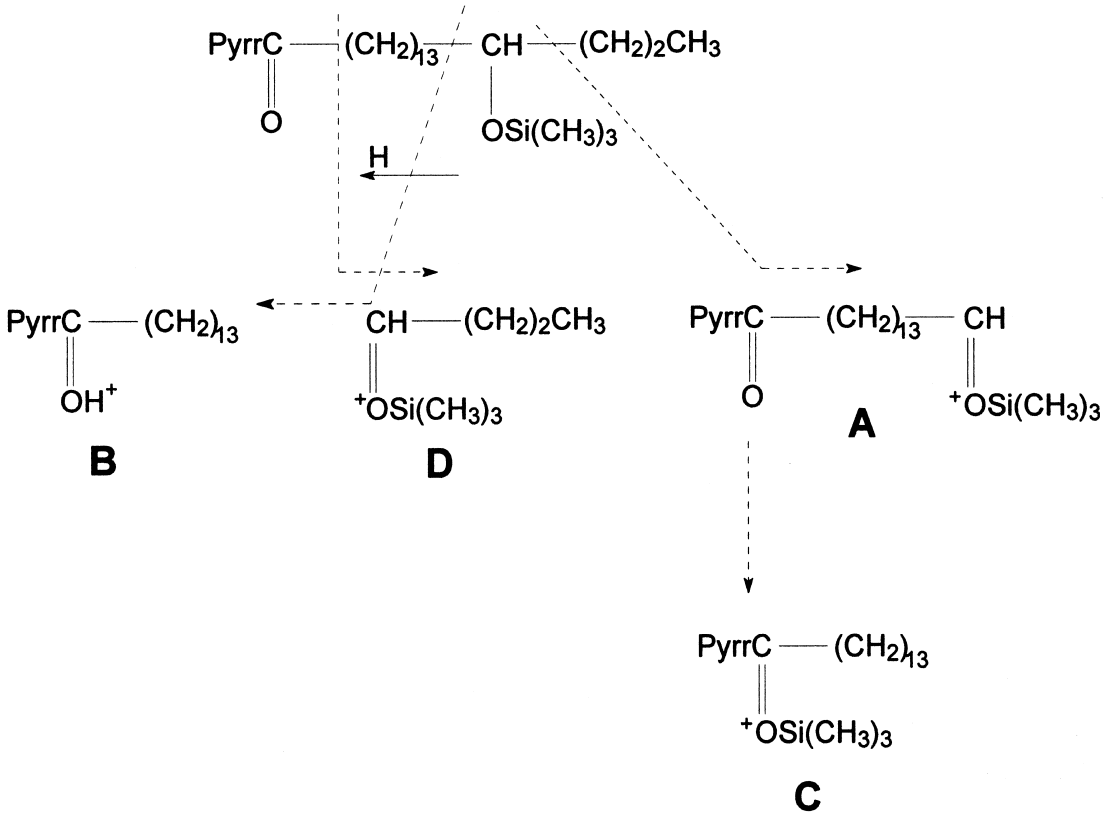


Fig. 2. Fragmentation of trimethylsilyl ether of 15-hydroxyoctadecanoic pyrrolidide.

by application of Lowe's rule (Lowe, 1965). Consequently, the configuration of the new C-18 bromoallenic acid with the two chiral centres at C-15 and C-18 was assigned as illustrated in formula **1**.

Compound **2**, $C_{18}H_{29}BrO_3$, mp 147°, $[\alpha]_D$ −211 exhibited those ^1H NMR spectral resonances expected for a disubstituted allene (δ 5.55 (1H, *dd*, $J=5.8$, 5.7 Hz) and 6.01 (1H, *dd*, $J=5.7$, 1.6 Hz)), (see Table 3), and in addition, the expected ^{13}C NMR chemical shifts (200.6, 102.8 and 76.3) as well as the ^{13}C–H coupling of the two remaining allenic carbon atoms ($J^{13}C\text{-}H_{16}=169$, $J^{13}C\text{-}H_{18}=211$ Hz). Observation of a weak allenic stretching band at 1945 cm^{-1} in the infrared spectrum supported this assignment. In addition, the mass spectral values *m/z* 358, 360 $[M+NH_4]^+$, 234 $[M+NH_4\text{-}C_3H_2Br]^+$ established the presence of a bromoallene group. Compound **2** was also established to have an additional *E* double bond [δ5.62 (1H, *ddd*, $J=15.7$, 6.4, 3.7 Hz) and 5.75 (1H, *dd*, $J=15.7$, 8.6 Hz); δ 134.9 and 127.8]. It thus has a total of three double bond equivalents (two in the bromoallene moiety and the *E* double bond) and is therefore obviously a long-chain triunsaturated fatty acid.

As indicated by the downfield carbon at δ 76.3 and by chemical shifts of H-15, i.e. δ 4.42, and also by the neighboring hydrogens (H-14 and H-16), compound **2** contains a hydroxyl group. The configuration of the hydroxyl group was determined by the modified method of Mosher (Ohtani et al., 1991; Rieser et al., 1992) and by the fact that full hydrogenation of **2** yielded compound **2a**. This compound was methylated and silylated and the appropriate ester-ether presented the same ions as compound **1a**.

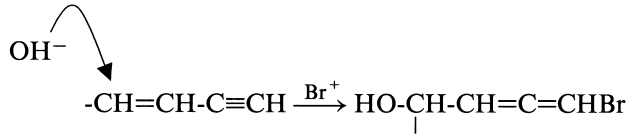
The following partial structure was established by detailed proton spin decoupling experiments:

$$HOOC-(CH_2)_{10}-CH_2-CH=CH-CHX$$
$$-CH=C=CHX \text{ where } X=O \text{ or } Br$$

The application of Lowe's rule (Lowe, 1965) to the bromoallenic acid (**2**) allows a tentative assignment of the absolute configuration of the allene moiety. According to this rule, unsymmetrically substituted allenes with the absolute configuration given in Fig. 1 show negative rotations at the sodium D line. The strong negative rotation observed for **2** $[\alpha]_D^{21}$ −211 corresponds to the configuration shown in Fig. 1. On the basis of the above-mentioned data, the structure of **2** depicted in Fig. 1 was established.

While the bromoallenic acids **1** and **2** have considerable novelty as natural halo-allenes, their partial structure indicating that they are closely related to the large group of halogenated acetylenic natural products of marine origin, all of which are based on sesquiterpenes and acetogenins with C15 skeletons. Representatives of this group have been found chiefly in red algae, but they also occur in sea hares (Grible, 1996). It has been suggested that

brominated marine terpenoids owe their biogenesis to an electrophilic bromination. It seems reasonable to postulate that bromoallenic acid is biosynthesized from a precursor bearing the typical enyne terminus via an analogous process, as shown below.



The acid F (6-bromo-(5*E*,15*Z*)-octadeca-5,15-diene-11,13,17-triynoic acid) from our previous paper (Řezanka and Dembitsky, 1999a) has a terminal enyne system and can serve as precursor of the above-mentioned bromoallenic acids (**1** and/or **2**).

Even though fatty acids with an allenic moiety have been known in the nature for a long time (Aitzetmüller et al., 1997; Spitzer et al., 1997) as indeed are halogenated fatty acids (Grible, 1996), this paper describes the first bromoallenic fatty acids from natural sources.

3. Experimental

The $CHCl_3$ extracts were obtained by the method of Blight and Dyer (1959) from the following lichens: *Acarospora gobiensis* H. Magn.; *Cladonia furcata* (Huds.) Schrad.; *Lecanora fructulosa* (Dicks.) Ach.; *Leptogium saturninum* (Dicks.) Nyl.; *Peltigera canina* (L.) Willd.; *Rhizoplaca peltata* Ram.; *Xanthoparmelia camtschadalis* (Ach.) Hale; *Xanthoparmelia tinctina* (Maheu & Gill.) Hale; and *Xanthoria elegans* (Link.) Th. Fr. All nine lichen species were extracted independently and the extracts were also chromatographed separately by means of the Sephadex LH-20 column with chloroform-hexane 65:35 and then separated by RP-HPLC (Supelco, RP-18, 85% MeOH-H_2O). This afforded compounds **1** and **2** (0.7–5.7 mg from 100 g of lichen, see Table 1).

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 (Micromass, Manchester, U.K), (positive chemical ionization mode with NH_3, source temperature 130°C). The ionizing condition for CI was 70 eV. GC–MS was performed using a Finnigan 1020 B instrument in EI mode. A fused silica capillary column (60 m×0.32 mm, ID/SPB-1 Supelco), using splitless injection and hydrogen as the carrier gas (a linear velocity of 600 mm/s) was used. The temperature program was 100–320°C, at a gradient of 4°C/min, ionization energy was 70 eV. All spectra were scanned within the range of m/z 50–600.

3.1. Catalytic hydrogenation

About 1 mg of the compounds (**1** and/or **2**) was dissolved in 1 ml of dry EtOH and a catalytic amount of PtO_2 was added. The mixture was stirred under H_2 for 2 h, the solution filtered and the EtOH evaporated to give, after chromatography on TLC (hexane-diethyl ether-acetic acid, 70:30:3), the pure compounds (**1a** and/or **2a**).

3.2. HPLC of enantiomers of 15-hydroxyoctadecanoic acid

The methyl esters were prepared by treating the fatty acid with an excess of ethereal diazomethane for 5 min at room temperature. The methyl esters were then further derivatized by reaction with 25 μl of 1-naphthoyl chloride in 30 μl of anhydrous pyridine and the reaction mixtures were incubated for 40 min at 60°C. The reaction mixtures were evaporated to dryness under a stream of nitrogen and the aromatic ester derivates extracted into hexane. Naphthoylated methyl esters were chromatographed on a DNBPG chiral stationary phase HPLC

Table 1
Occurrence of bromoallenic acids from lichens of Tian Shan mountains

Name of lichen	Name of compounds (mg/100 g of dry weight)	
	Compound **1**	Compound **2**
Acarospora gobiensis	2.8	0.7
Cladonia furcata	–[a]	1.9
Lecanora fructulosa	3.2	–
Leptogium saturninum	2.5	–
Peltigera canina	–	1.8
Rhizoplaca peltata	5.7	–
Xanthoparmelia camtschadalis	–	3.6
X. tinctina	–	0.7
Xanthoria elegans	1.5	–

[a] Less than 0.1 mg/100 g of dry weight.

Table 2
^{13}C NMR of compounds **1** and **2**

Carbon no.	Compound **1**	Compound **2**
1	174.2 *s*	176.1 *s*
2	34.4 *t*	34.3 *t*
3	25.9 *t*	25.3 *t*
4–10	28.7–29.4	28.8–29.4
11	26.4 *t*	29.3 *t*
12	127.0 *d*	31.9 *t*
13	132.1 *d*	127.8 *d*
14	40.0 *t*	134.9 *d*
15	74.4 *d*	76.3 *d*
16	102.4 *d*	102.8 *d*
17	201.9 *s*	200.6 *s*
18	74.1 *d*	73.6 *d*

Table 3
^{1}H NMR of compounds **1** and **2**

Hydrogen	Compound **1**	Compound **2**
2	2.24 (2H, *m*)	2.19 (2H, *m*)
3	1.55 (2H, *m*)	1.57 (2H, *m*)
4–10	1.28-1.35 (16H, *br s*)	1.28 -137 (16H, *br* s)
11	1.96 (2H, *m*)	1.30 (2H, *m*)
12	5.39 (1H, *ddd*, $J_{12,13}=9.7$, $J_{12,11a}=6.2$, $J_{12,11b}=7.1$)	2.02 (2H, *m*)
13	5.46 (1H, *ddd*, $J_{13,14a}=0.6$, $J_{13,14b}=5.7$, $J_{13,12}=9.7$)	5.62 (1H, *ddd*, $J_{13,12a}=3.7$, $J_{13,12b}=6.4$, $J_{13,14}=15.7$)
14a	2.48 (1H, *ddd*, $J_{14a,14b}=13.2$, $J_{14a,15}=10.0$, $J_{14a,13}=0.6$)	5.75 (1H, *dd*, $J_{14,13}=15.7$, $J_{14,15}=8.6$)
14b	2.01 (1H, *ddd*, $J_{14b,14a}=13.2$, $J_{14b,15}=5.0$, $J_{14b,13}=5.7$)	
15	4.86 (1H, *dddd*, $J_{15,16}=6.0$, $J_{15,18}=1.5$ $J_{15,14a}=10.0$, $J_{15,14b}=5.0$)	4.42 (1H, *ddd*, $J_{15,14}=8.6$, $J_{15,16}=5.8$,$J_{15,18}=1.6$)
16	5.51 (1H, *dd*, $J_{16,15}=6.0$, $J_{16,18}=5.5$)	5.55 (1H, *dd*, $J_{16,15}=5.8$, $J_{16,18}=5.7$)
18	6.09 (1H, *dd*, $J_{18,15}=1.5$, $J_{18,16}=5.5$)	6.01 (1H, *dd*, $J_{18,15}=1.6$, $J_{18,16}=5.7$)

column, (5 μm modified Chirex, 25×4.6 mm i.d. obtained from Phenomenex, CA, USA). The derivatives were eluted with a mobile phase solvent system of *n*-hexane containing 0.5% 2-propanol (v/v); the products were eluted isocratically at a flow rate of 0.5 ml per minute. The UV absorbance of the effluent was monitored at 235 nm.

The resolution α for both *R* and *S* isomers of 15-hydroxyoctadecanoic acids was calculated according to the following formula: $\alpha = k'/k$, where k is the capacity factor of the first eluting peak and k' the capacity factor of the second eluting peak.

3.3. GC–MS of TMS ethers of pyrrolidides of 15-hydroxy octadecanoic acid

Glacial acetic acid (0.1 ml) was added to pyrrolidine (1 ml). The mixture was cooled to 0°C, methyl 15-hydroxyoctadecanoate (~1 mg) was added and the solution was kept at 25°C for 24 h. The solution was poured into water, the product extracted with $CHCl_3$ and the extract washed with 1 M HCl and with water. TMS ethers were prepared by treating the hydroxy pyrrolidides in CH_2Cl_2 with bis(trimethyl)silyl acetamide.

Mass spectrum of trimethylsilyl ether of 15-hydroxyoctadecanoic pyrrolidide: MS *m*/*z* (rel. int.) 425 $[M]^+$ (4), 410 $[M-15]^+$ (21), 382 $[D]^+$ (74), 353 $[C]^+$ (3), 335 $[M-90]^+$ (5), 281 $[B]^+$ (9), 145 $[A]^+$ (68), 113 (100).

(12*Z*,15*S*,18*S*)-15-Hydroxy-18-bromo-12,16,17-octadecatrienoic acid (1), *m*p 129°C; $[\alpha]_D^{21}$ +142°; IR (as neat film) 3400, 1958 and 1710 cm^{-1} ; MS-CI (NH_3, *m*/*z*) $(M+NH_4)^+$ 358 and 360 (intensity ratio 1:0.97)., $(M+NH_4\text{-}C_3H_2Br)^+$ 234; HR-MS-PCI (NH_3, *m*/*z*) calcd for $(C_{18}H_{29}O_3{}^{79}Br+NH_4)^+$ 373.3304, found 373.3308.

(13*E*,15*S*,18*S*)-15-Hydroxy-18-bromo-13,16,17-octadecatrienoic acid (2), *m*p 147°C; $[\alpha]_D^{21}$ −211°; IR (as neat film) 3400, 1945 and 1715 cm^{-1} ; MS-CI (NH_3, *m*/*z*) $(M+NH_4)^+$ 358 and 360 (intensity ratio 1:0.98)., $(M+NH_4\text{-}C_3H_2Br)^+$ 234; HR-MS-PCI (NH_3, *m*/*z*) calcd for $(C_{18}H_{29}O_3{}^{79}Br+NH_4)^+$ 373.3304, found 373.3306.

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