Pergamon

S0031-9422(96)00163-X

Phytochemistry, Vol. 42, No. 6, pp. 1683–1687, 1996

0031-9422/96 $15.00 + 0.00

THREE OXANTHRONES FROM *ALOE LITTORALIS*

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(*Received* 3 *January* 1996)

Key Word Index—*Aloe littoralis*; Aloaceae; C-glucoside oxanthrone; nilic acid; methyl nilate; littoralin; deacetyllittoralin; 10-hydroxyaloin B.

Abstract—The leaf exudate of *Aloe littoralis* yielded, in addition to the known 10-hydroxyaloin B, two new C-glucoside oxanthrones namely 10-hydroxy-15-*O*-(2*R*,3*S*-nilyl)(6′-*O*-acetyl)aloin B (littoralin) and 10-hydroxy-15-*O*-(2*R*,3*S*-nilyl)aloin B (deacetyllittoralin). Their structures were determined by spectroscopic methods as well as by conversion to the known 10-hydroxyaloin B and nilic acid.

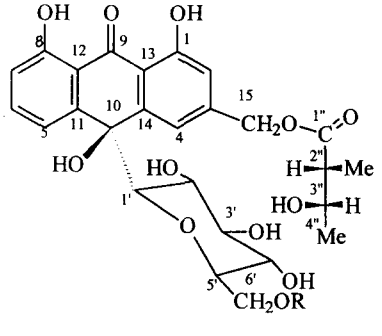
INTRODUCTION

Aloe littoralis Bak. is a simple stemmed shrub which is widely distributed in southern Africa including Angola, Mozambique, Zimbabwe, Namibia and South Africa [1]. On investigation of the crude leaf exudates of a variety of *Aloe* species by HPLC and TLC, we noted the absence in *A. littoralis* of the common constituent of aloe drugs namely aloin (also known as barbaloin). Instead, the presence of four other polar components was detected. We report here on the structure elucidation of three of these compounds namely the new natural products littoralin (**1**) and deacetyllittoralin (**2**) and the closely related known compound 10-hydroxyaloin B (**3**).

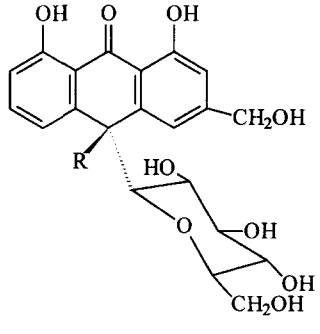
RESULTS AND DISCUSSION

TLC analysis of the leaf exudate of *A. littoralis* showed three yellow spots with R_f values of 0.5, 0.7 and 0.8 ($CHCl_3$–MeOH, 4:1). In the same system aloin has an R_f value of 0.5. The separation of these compounds was achieved by column chromatography over silica gel followed by PTLC. The spots with R_f values of 0.8 and 0.7 corresponded to the new compounds **1** and **2**, respectively, while the remaining spot, R_f0.5, was due to the known compound 10-hydroxyaloin B (**3**).

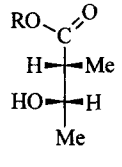
Littoralin (**1**) exhibited pseudomolecular ions at *m/z* 577 ($[M+H]^+$), and 599 ($[M+Na]^+$) in the positive-ion 575, ($[M-H]^-$) in the negative-ion FAB-



1 R = Ac
2 R = H



3 R = OH
6 R = H



4 R = H
5 R = Me

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of littoraloin (**1**) and deacetyllittoraloin (**2**) in Me_2CO-d_6 and 10-hydroxyaloin B (**3**) in MeOH-d_4

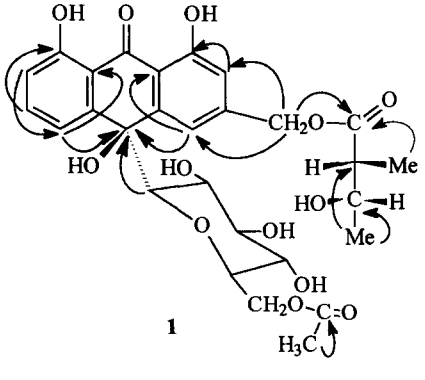
H	1	2	3
1-OH	11.80 *s*	11.87 *s*	11.76 *s*
8-OH	11.80 *s*	11.88 *s*	11.81 *s*
H_2-2	6.97 *d* (1.6)	6.95 *d*	6.87 *d*(1.5)
H_2-4	7.54 *d*(1.6)	7.55 *d*	7.40 *d*(1.5)
H_2-5	7.39 *dd*(8.0, 1.0)	7.40 *dd*	7.32 *dd*(7.8, 1.0)
H_2-6	7.59 *t* (8.0)	7.59 *t*	7.62 *t*(7.8)
H_2-7	6.95 *dd*(8.0, 1.0)	6.92 *dd*	6.93 *dd*(7.8, 1.0)
H_2-15	5.22 *s*	5.22 *s*	4.70 *s*
H_2-1′	3.22 *d*	3.26 d	3.23 d
H_2-2′	3.14 *t*	3.05 *t*	3.08 *t*
H_2-3′	3.05 *t*	3.36 *t*	3.38 *t*
H_2-4′	2.84 *t*	2.90 *t*	2.92 *t*
H_2-5′	3.40 *m*	3.00 *m*	2.98 *m*
H_2-6′$_1$	4.08 *dd*	3.52 *dd*	3.50 *dd*
H_2-6′$_2$	3.68 *dd*	3.32 *dd*	3.32 *dd*
H_2-2″	2.85 *m*	2.58 *m*	—
Me-2″	1.22 *d*(6.9)	1.22 *d*	—
H_2-3″	4.05 *m*	4.02 *m*	—
Me-4″	1.18 *d*(6.3)	1.18 *d*	—
OAc	1.90 *s*	—	—

mass spectra, indicating a M_r of 576, corresponding to the molecular formula $C_{28}H_{32}O_{13}$, consistent with the ^{13}C and ^{1}H NMR data. The IR spectrum of **1** suggested the presence of a hydroxyl (3387 cm^{-1}), ester carbonyl (1735 cm^{-1}) and chelated carbonyl group (1639 cm^{-1}).

The presence of 28 carbons was evident from the ^{13}C NMR spectrum. The DEPT experiment revealed three methyls (δ 12.5, 20.4, 21.5), two oxymethylenes (δ 64.2, 65.6), 12 methines and 11 quaternary carbons including three carbonyls. The presence of two chelated hydroxyl groups was supported by the ^{1}H NMR spectrum which showed a broad singlet at δ 11.80, in addition to five aromatic protons assignable to H-2 (δ 6.97, *d*, J = 1.6 Hz), H-4 (δ 7.54, *d*, J = 1.6 Hz), H − 5 (δ 7.39, *dd*, J = 8.0, 1.0 Hz), H-6 (δ 7.59, *t*, J = 8.0 Hz) and H-7 (δ 6.95, *dd*, J = 8.0, 1.0 Hz) and other signals as shown in Table 1.

Correlation between proton and carbon resonances was achieved by means of HETCOR and COLOC connectivities. Decisive observations from the COLOC (Fig. 1) and selective decoupling experiments were as follows: (i) A ^3J coupling between the aromatic protons H-2 and H-4 to the oxymethylene carbon at δ 65.6 indicated the linkage of the oxymethylene group to C-3. Likewise, C-2 and C-4 couple with the oxymethylene protons. (ii) The protons of the oxymethylene (δ 5.22, *s*) gave rise to a cross peak with the ester carbonyl at δ 175.1 showing the presence of an ester linkage at C-15. This carbonyl showed further cross-peaks with a methine proton (δ 2.85, *m*, H-2″) and the methyl protons at δ 1.22. Conversely the methyl protons at δ 1.18 (H-4″) showed two cross-peaks, to a methine carbon at δ 47.7 (C-2″) and the oxymethine carbon at δ 68.9 (C-3″). These observations along with the FAB-mass spectral data which showed (Fig. 2) loss of a molecule of water from the protonated molecular ion resulting first in an intense fragment at *m/z* 559, and subsequent loss of a fragment with mass 117 to yield an ion of *m/z* 442 allowed assignment of a nilate (3-hydroxy-2-methylbutanoate) structure for the ester group attached to C-15. (iii) A ^3J coupling between the aromatic protons, H-4 and H-5 to the quaternary carbon at δ 76.9 (C-10), which also correlated with H-1′ (δ 3.22, *d*) of the glucose moiety. (iv) The coupling of the remaining methyl protons (δ 1.90, *s*) with the other carbonyl resonating at δ 170.8 indicated the presence of an acetate moiety in compound **1**.

The acetate group was placed on the C-6′ hydroxyl group of the glucose moiety due to the downfield shift of the C-6′-methylene protons (δ 3.68, 4.08) in comparison to those in compounds **2** and **3** (δ 3.32, 3.52). Separate irradiation of the resonances of the nonequivalent methylene protons of the glucose of **1** in the proton-coupled ^{13}C NMR spectrum resulted in each case in the collapse of the multiplet of the acetate carbonyl carbon to a doublet of a quartet. The ^3J coupling between the acetate carbonyl and the 6′-methylene protons was found to be 2.0 and 2.4 Hz, while the ^2J coupling with the methyl was 6.7 Hz. It is interesting to note that in the above-described COLOC



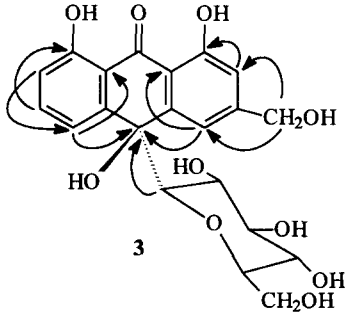
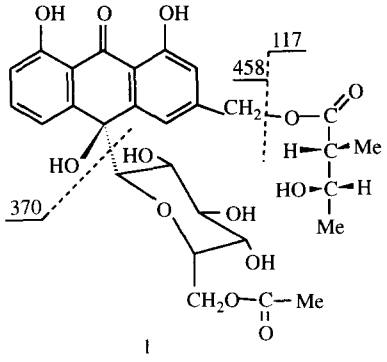
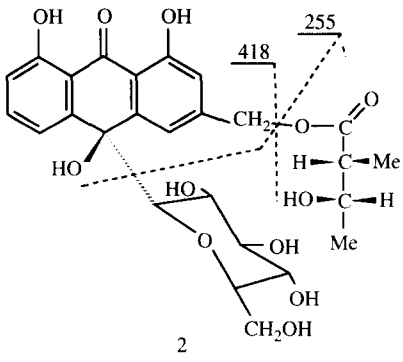


Fig. 1. Selected two-dimensional COLOC correlations for littoraloin (**1**) and 10-hydroxyaloin B (**3**).



1



2

Negative FAB	$[M - H]^-$ = 575	Negative FAB	$[M + Na]^+$ = 557
	$[M - H\text{-nilate}]^-$ = 458		$[M + H]^+$ = 535
	$[M - H\text{-GlcOAc}]^-$ = 370		$[M + H\text{-}H_2O]^+$ = 517
	$[M - H\text{-GlcOAc-nilate}]^-$ = 253		$[M + H\text{-nilate}]^+$ = 418
	$[\text{nilate}]^-$ = 117		$[M + H\text{-nilate-Glc}]^+$ = 255
Positive FAB	$[M + H]^+$ = 577	Positive FAB	$[M - H]^-$ = 533
	$[M + Na]^+$ = 599		$[M - H\text{-nilate}]^-$ = 416
	$[M + H\text{-}H_2O]^+$ = 559		$[M - H\text{-nilate-Glc}]^-$ = 253
	$[M + H\text{-GlcOAc}]^+$ = 372		
	$[M + H\text{-GlcOAc-nilate}]^+$ = 255		

Fig. 2. Fragmentation pattern of compounds **1** and **2** in FAB-mass spectrometry.

spectrum no correlation was observed between these methylene protons and the acetate carbonyl because the COLOC measurement was optimized for a typical coupling of 7 Hz.

Thus, the ^1H and ^{13}C NMR data presented in Tables 1 and 2, respectively, which have been corroborated by the COLOC, HETCOR and COSY data, indicated compound **1** to have an aloin skeleton with hydroxy substituent on C-10, acetyl at C-6′ of the glucose and nilyl on the oxygen of C-15.

The stereochemistry at C-10 and that of the nilate moiety was deduced as follows. The ^1H and ^{13}C NMR spectral data of the product obtained by mild acid hydrolysis of compound **1** (see Experimental) indicated loss of the acetyl group. The product was in every respect identical to compound **2**, which is also present in the extract of *A. littoralis*. Further hydrolysis of **1** and also of **2** led to the isolation of the C-glucoside **3**, in which both the ester groups present in **1** were removed. Thus, compound **3** was identical in all respects to the third constituent of the plant extract.

The positive FAB-mass spectrum of **3** gave a $[M + H]^+$ ion at *m/z* 435 while the negative FAB-mass spectrum yielded $[M - H]^-$ at *m/z* 433 and $[M - H - Glc]^-$ at *m/z* 270. From this and the ^1H and ^{13}C NMR spectral data (Tables 1 and 2, respectively) the molecular formula $C_{21}H_{22}O_{10}$ could be deduced, and structure **3** followed from analysis of the two-dimensional NMR data, including COLOC correlations as depicted diagrammatically in Fig. 1. Compound **3** is thus the

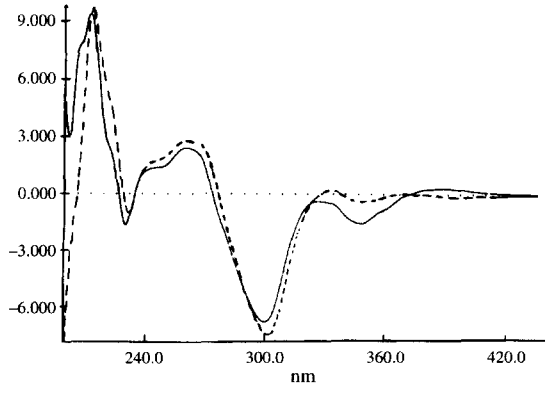


Fig. 3. CD spectra in methanol of 10-hydroxyaloin B (– – –) and littoraloin (—).

Table 2. ^{13}C NMR spectral data of littoraloin (**1**) and deacetyllittoraloin (**2**) in Me_2CO-d_6 and 10-hydroxyaloin B (**3**) in MeOH-d_4

C	1	2	3
1	162.1	162.2	162.3
2	115.7	115.7	114.5
3	149.4	149.1	152.9
4	115.9	116.2	115.4
5	118.4	118.7	118.1
6	136.1	136.3	136.1
7	117.8	117.7	117.7
8	162.5	162.5	162.5
9	194.0	194.2	194.2
10	76.9	76.5	76.7
11	145.9	146.4	146.5
12	117.2	117.1	117.2
13	116.6	116.3	115.6
14	146.5	146.5	148.8
15	65.6	64.3	64.2
1′	83.4	84.1	84.1
2′	72.6	72.7	72.7
3′	78.3	79.1	79.1
4′	71.0	71.5	71.5
5′	78.9	81.0	80.9
6′	64.2	63.0	63.0
1″	175.1	175.1	–
2″	47.7	47.6	–
3″	68.9	68.6	–
4″	21.5	21.5	–
2″-Me	12.5	12.2	–
$O\underline{C}CH_3$	170.8	–	–
$OC\underline{C}H_3$	20.4	–	–

recently reported 10-hydroxyaloin B [2]. The α-orientation of the glucose group at C-10 in **3** was confirmed by the identity of the CD spectrum with that reported for the same compound by Rauwald *et al.* [2]. Furthermore, as pointed out by these authors, in the ^1H NMR spectrum of compound **3**, H-4 (*d*) appeared downfield in comparison with H-5 (*dd*); in the case of 10-hydroxyaloin A, the reverse ordering has been reported [3]. Therefore, because the CD spectrum of compound **1** was nearly superimposable on that of compound **3**, the α-orientation could be assigned to the glucose group in **1**.

From the above hydrolysis reaction, it was possible to isolate an acid which was identified as nilic acid (**4**) from its ^1H and ^{13}C NMR spectral data, and further confirmed by converting it to its methyl ester (5) and comparing the spectral data of this derivative with the data in the literature [4–7]. The absolute stereochemistry of the nilate moiety was assigned by comparison of the specific rotations of methyl nilate (**5**) with reported values for the stereo isomers of this compound in the literature (Table 3).

Furthermore, Hoffmann *et al.* [6] reported the specific rotations ($[\alpha]_\lambda$(MeOH; *c* 5.63)) of (+)-erythro methyl nilate at various wavelengths (λ(nm): 589, +8.70; 578, +9.24; 546, +9.95; 436, +17.94; 365, +30.37. The specific rotation values ($[\alpha]_\lambda$(MeOH; *c* 2.1)) of methyl nilate (**5**) obtained by methylation of

Table 3. Specific rotations reported for the stereoisomers of methyl nilate (**5**) in the literature

Isomer	Absolute configuration	$[\alpha]_D$	Ref.
(+)-threo	(2*S*,3*S*)	+36.8	[4]
(+)-threo	(2*S*,3*S*)	+27.8	[7]
(−)-threo	(2*R*,3*R*)	−34.7	[4]
(−)-threo	(2*R*,3*R*)	−30.8	[5]
(+)-erythro	(2*S*,3*R*)	+14.3	[4]
(+)-erythro	(2*S*,3*R*)	+8.7	[6]
(−)-erythro	(2*R*,3*S*)	−11.4	[4]

nilic acid derived from acid hydrolysis of **1** and **2** were as follows: (λ(nm)): 589, −10.1; 578, −10.6; 546, −12.2; 436, −22.4; 365, −22.7.

These results indicate that the nilate moiety present in compounds **1** and **2** has the (−)-erythro, i.e. (2*R*,3*S*) configuration. Compound **1** represents a new *C*-glucoside oxanthrone, 10-hydroxy-15-*O*-(2*R*,3*S*-nilyl) (6′-*O*-acetyl)aloin B for which the trivial name littoraloin is proposed.

Deacetyllittoraloin (**2**), which could also be obtained by mild acid hydrolysis of **1**, was assigned the molecular formula $C_{26}H_{30}O_{12}$ based on the pseudomolecular ions at *m/z* 535 ($[M+H]^+$) and 557 ($[M+Na]^+$) in the positive-ion and at *m/z* 533 ($[M-H]^-$) in the negative-ion FAB-mass spectra. The ^1H and ^{13}C NMR data of compound **2** (see Tables 1 and 2) including COLOC correlations showed that it differs from compound **1** only by the lack of the acetyl group. Furthermore, the α-orientation of the glucose substituent at C-10 was confirmed by the close correspondence of the CD spectrum of compound **2** with that known for 10-hydroxyaloin B [2]. These results led to structure **2**, which is 10-hydroxy-15-*O*-(2*R*,3*S*-nilyl)aloin B, named deacetyllittoraloin.

10-Hydroxyaloin A and B were reported as constituents of *Aloe* and *Rhamnus* species [2] and were also obtained by oxidation of the respective diastereomeric aloins A and B [3]. In the case of the aloins it has been pointed out [8] that only the 10α-diastereomer (i.e. aloin B) is formed in the plant, and later this epimerizes at C-10 to aloin A. It is interesting to note that the α-linkage of the glucose moiety to C-10 of the anthrone remains unaltered in compounds **1**, **2** and **3**, suggesting that aloin B (**6**) is the most likely precursor.

EXPERIMENTAL

General. Mps: uncorr.; Optical rotation: MeOH; UV: MeOH; IR: KBr discs and $CHCl_3$; ^1H NMR (Varian VXR 400 S, 400 MHz and Bruker AMXR 300, 300 MHz) and ^{13}C NMR (Varian VXR 400 S, 100 MHz and Bruker AMXR 300, 75 MHz); DMSO-d_6 and Me_2CO with TMS as int. standard; FAB-MS (Finnigan MAT 95Q double focusing MS with caesium gun): glycerine and dimercaptobutandiol as matrices; CI-MS: Isobutane; TLC solvent system I: $CHCl_3$–MeOH (4:1).

Plant material. The leaves of *A. littoralis* were collected at Vivo in northern Transvaal, near the western end of the Soutpansberg, South Africa and identified by B-E. Van Wyck and M. C. B. van Oudtshoorn. A voucher specimen is deposited at the Rand Afrikaans University Herbarium.

Extraction and isolation. The leaf of *A. littoralis* (50 g) was taken up in MeOH. The MeOH extract was concd and the residue was subjected to flash chromatography over silica gel eluting with EtOAc and MeOH gradients. The frs were further purified by CC on Sephadex LH-20 (MeOH) which resulted in the isolation of pale yellow substances: **1** (400 mg), **2** (300 mg) and **3** (600 mg).

Littoraloin (**1**). Yellow amorphous, $[\alpha]_D$ − 26.0 (MeOH; *c* 1.0), R_f 0.8 (Solvent I). UV λ_{max} nm: 270, 300, 370; IR υ_{max} cm^{-1}: 3387, 2976, 1735, 1639, 1617, 1576, 1489, 1453; ^1H NMR: (Table 1; ^{13}C NMR: Table 2; FAB-MS (matrix: glycerine): *m/z* 599 $[M + Na]^+$, 577 $[M + H]^+$, 559 $[M + H - H_2O]^+$, 500 $[M + H - H_2O - OAc]^+$, 442 $[M + H - H_2O$-nilate$]^+$, 370 [M−Ac−Glc]$^+$, 254 [M−nilate−Ac−Glc]$^+$.

Deacetyllittoraloin (**2**). Yellow amorphous, $[\alpha]_D$ −37.9 (MeOH; *c* 1.55), R_f 0.7 (Solvent I). UV λ_{max}nm: 270, 300, 370; IR $\upsilon_{max}$$cm^{-1}$: 3373, 2975, 2928, 1734, 1639, 1619, 1576, 1489, 1452, 1370; ^1H NMR: Table. 1, ^{13}C NMR: Table. 2; FAB-MS: *m/z* 557 $[M + Na]^+$, 535 $[M + H]^+$, 517 $[M + H - H_2O]^+$, 372 [M + H−Glc]$^+$, 255 [M + H−nilate−Glc]$^+$.

10-*Hydroxyaloin B* (**3**). Yellow solid, mp 136–138°, $[\alpha]_D$-52.3 (MeOH; *c* 1.0), R_f 0.5 (Solvent I). UV λ_{max} nm: 270, 300, 370; IR υ_{max} cm^{-1}: 3355, 2920, 1637, 1613, 1574, 1486, 1452, 1365; ^1H NMR: Table 1; ^{13}C NMR: Table 2; FAB-MS: *m/z* 435 $[M + H]^+$, 417 $[M + H - H_2O]^+$, 272 $[M + H - Glc]^+$, 255 $[M + H - Glc - OH]^+$.

Mild hydrolysis of compound **1**. To compound **1** (1 mg), dissolved in MeOH (4 ml) was added 2N HCl (1 ml) and the mixture stirred overnight. TLC analysis showed complete disappearance of compound **1** and the formation of compounds **2** and **3** (co-TLC).

Strong hydrolysis of compounds **1** *and* **2**. Compound **1** (200 mg) was dissolved in 2.5% KOH in MeOH H_2O (3:2) and the soln was stirred overnight. The solvent was removed, acidified, satd with NaCl and extracted with EtOAc to yield **3** (^1H and ^{13}C NMR and co-TLC). The aq. phase was acidified and extracted with EtOAc to give 50 mg of nilic acid (**4**).

Methylation of **4**. Compound **4** (50 mg) was methylated using CH_2N_2 in Et_2O at 0°. The reaction mixt. was kept in ice for 30 min and then allowed to stand overnight at room temp. and this resulted in **5** (40 mg) which was identified as methyl (2*R*,3*S*)-nilate.

Methyl (2R,3S)-*nilate* (**5**). $[\alpha]_D$ − 10.1 (MeOH; *c* 2.1). IRυ_{max} cm^{-1}: 3417, 3018, 1725, 1458, 1215, 1087; ^1H NMR (300 MHz, $CDCl_3$): δ 4.05 (1H, *m*), 3.67 (3H, *s*), 2.46 (1H, *m*), 1.16 (3H, *d*), 1.13 (3H, *d*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.3 (C-1), 67.9 (C-3), 51.7 (OCH_3), 45.7 (C-2), 19.8 (4-Me), 11.0 (2-Me); CI-MS: *m/z* 133 $[M + H]^+$, 117 $[M - OH]^+$, 101 $[M - OCH_3]^+$, 88 $[M - CH_3CHO]^+$.

Acknowledgements—Daniel Bisrat is thanked for technical assistance and helpful discussions. E.D. is grateful to the Alexander von Humboldt Foundation (FRG) for a fellowship. Rita Fröde is thanked for FAB-mass spectrometry measurements.

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