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ISOMERIC TRILUTEOLINS FROM *BARTRAMIA STRICTA* AND *BARTRAMIA POMIFORMIS**

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Key Word Index—*Bartramia stricta*; *B. pomiformis*; Bartramiaceae; Musci; bartramiatriluteolin; epibartramiatriluteolin; strictatriluteolin; triluteolins; triflavones.

Abstract—From *Bartramia stricta* and *B. pomiformis* the new triluteolins epibartramiatriluteolin and strictatriluteolin along with the known bartramiatriluteolin were isolated. The structures and relative stereochemistry of these triluteolins have been elucidated spectroscopically.

INTRODUCTION

As stated in a preceding paper on bi- and tri-flavonoids from *Bartramia stricta*, this moss contained in addition to the reported flavonoids a mixture that consists, according to its NMR spectrum, of at least three further triflavones [1]. A small amount of a similar mixture had also been obtained earlier from *B. pomiformis* [2]. After many unsuccessful attempts the separation of this mixture has now been achieved by column chromatography on polyamide and on Sephadex LH-20 at low temperature (see Experimental). In the present paper the elucidation of the structures of the new compounds is described.

RESULTS AND DISCUSSION

The first compound, which was later named epibartramiatriluteolin (**1b**), was separated from the bulk of the mixture by column chromatography on polyamide. The relative molecular mass of **1b** is, as required for a triluteolin, 854 amu ([M − 1] anion at 853 *m/z* in the LAMMA mass spectrum). The NMR data are presented in Table 1. The ^{13}C NMR spectrum of **1b** differs only marginally from that of bartramiatriflavone (**1a**), which occurs also in *B. pomiformis* [3]. The coupling patterns of the ^{1}H NMR spectra of **1a** and **1b** are also the same, only the chemical shifts of the proton signals of the two compounds differ markedly. A comparison of the spectra of **1a** and **1b** with a set of C–H correlated NMR spectra of various biluteolins [4] indicates clearly that in both cases the three luteolin moieties are connected via the carbon atoms IB2′, IIA8, IIB2′ and IIIA8 (Fig. 1). This allows two possibilities of interflavonyl linkages: Either IB2′ → IIA8 and IIB2′ → IIIA8 or IB2′ → IIB2′ and IIA8 → IIIA8. The latter possibility can be excluded, because the resonances of the bridgehead carbon atoms of carbon–carbon linked biflavonoids are shifted downfield from their position in the corresponding monomer (naringenin, apigenin, eriodictyol, taxifolin, luteolin or aureusidin) to a different extent, whether the interflavonyl linkage is between two A-rings, two B-rings or an A- and a B-ring. As a rule this shift is about 4.5–6.0 ppm if the linkage is between two A-rings and 10.1–11.4 ppm if it is between two B-rings (*e.g.* see refs [5] and [6, 7], respectively). If, however, the interflavonyl linkage is between an A- and a B-ring the situation is quite different (*e.g.* see refs [3, 8–11]): In this case the signals of the A-ring bridgehead carbon atoms are *ca* 9–11 ppm downfield of their position with the corresponding monomers, and the downfield shift of the B-ring bridgehead carbon signals is only 4–6.5 ppm. This is the case with **1a** as well as **1b**, which are therefore both linked IB2′ → 8IIA and IIB2′ → 8IIIA. Thus, the reason for the different chemical shifts exhibited by the ^{1}H NMR spectra of **1a** and **1b** must be caused by a different steric arrangement of the three luteolin moieties with the two compounds. It is known that the rotation round a carbon–carbon interflavonyl bond between a 2′ and an 8 position of flavonoids is sterically hindered. Biflavonoids having such interflavonyl linkages show atropisomerism [12]. With triflavonoids having two such linkages the existence of two diastereomers is to be expected. Figure 2 shows stereo-drawings of the two possible diastereomers; they reveal at a glance which formula belongs to which ^{1}H NMR spectrum: Formula **1a**, which has its

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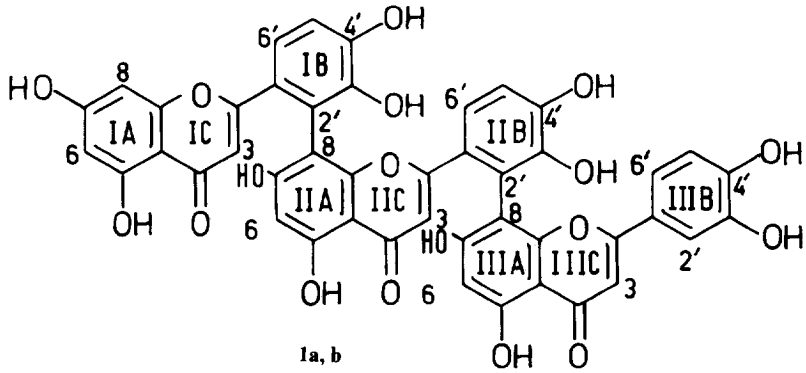
Table 1. ^1H and ^{13}C NMR data of bartramiatriluteolin (**1a**), epibartramiatriluteolin (**1b**), and the rotamere mixture of strictatriluteolin (**2**)*

		1a		**1b**		**2**			
		^1H†	^{13}C	^1H	^{13}C	^1H (500 MHz)		^{13}C (125 MHz)	
	Position	(400 MHz)	(100 MHz)	(200 MHz)	(50 MHz)	Major	Minor	Major	Minor
	2		166.1		166.7	—	—	166.5	166.6
	3	5.72 *s*	105.7	5.91 *s*	106.1	5.73 *s*	5.97 *s*	106.2	106.7
	4		181.4		181.5	—	—	181.3	181.4
	5		161.7		161.3	—	—	161.3	161.2
	OH-5	12.85 *s*		12.83 *s*		12.69 *s*	12.71 *s*	—	—
	6	6.02 *dm*	98.2	6.11 *dm*	98.3	5.97 *dm*	6.06 *dm*	98.5	98.6
	7		163.5		164.1	—	—	163.8	164.0
	8	5.33 *dm*	93.0	5.75 *dm*	93.5	5.71 *dm*	5.75 *dm*	93.1	93.3
I	9		156.9		157.3	—	—	157.2	157.1
	10		103.2		103.0	—	—	103.3	103.3
	1′		123.8		123.7	—	—	123.8	123.7
	2′		118.4		118.7	—	—	118.8	118.9
	3′		143.9		144.1	—	—	144.0	144.1
	4′		148.0		148.4	—	—	148.0	148.2
	5′	6.91 *do*	114.3	6.97 *do*	114.5	6.92 *do*	6.98 *do*	114.3	114.6
	6′	7.10 *do*	120.8	7.17 *do*	120.8	7.12 *do*	7.24 *do*	120.6	120.7
	2		165.6		165.6	—	—	166.2	165.3
	3	5.74 *s*	106.4	5.60 *s*	106.1	5.89 *s*	5.79 *s*	107.0	106.2
	4		181.1		181.4	—	—	181.0	181.2
	5		160.0		160.4	—	—	159.1	158.6
	OH-5	12.86 *s*		12.86 *s*		12.79 *s*	12.86 *s*	—	—
	6	6.08 *s*	98.1	6.14 *s*	99.1	6.14 *s*	6.18 *s*	98.2	98.2
	7		161.0		161.2	—	—	161.0	161.2
	8		103.2		103.4	—	—	103.4	103.4
II	9		153.9		154.6	—	—	155.1	155.2
	10		103.4		103.5	—	—	103.5	103.5
	1′		124.4		123.8	—	—	124.4	123.8
	2′		118.9		121.9	—	—	119.7	119.8
	3′		144.2		144.2	—	—	144.2	144.3
	4′		148.2		148.6	—	—	148.2	148.5
	5′	6.86 *do*	114.3	6.85 *do*	114.3	6.79 *do*	6.76 *do*	114.0	114.0
	6′	7.04 *do*	119.9	7.12 *do*	120.7	6.90 *do*	6.92 *do*	120.2	120.2
	2		163.4		163.9	—	—	163.3	163.6
	3	6.19 *s*	102.0	6.61 *s*	102.7	6.50 *s*	6.57 *s*	102.6	103.0
	4		181.8		181.9	—	—	181.5	181.6
	5		160.6		160.6	—	—	160.2	160.3
	OH-5	13.01 *s*		12.98 *s*		12.95 *s*	13.10 *s*	—	—
	6	6.25 *s*	98.3	6.20 *s*	98.7	—	—	107.4	108.4
	7		160.6		161.1	—	—	160.3	161.1
	8		103.8		103.7	6.39 *s*	6.47 *s*	93.1	93.7
III	9		155.2		155.2	—	—	156.2	156.4
	10		102.5		102.4	—	—	103.1	103.1
	1′		121.1		121.9	—	—	121.4	121.5
	2′	6.68 *dm*	112.7	7.03 *dm*	113.7	7.26 *dm*	7.36 *dm*	113.1	113.3
	3′		145.1		145.6	—	—	145.5	145.7
	4′		148.9		149.6	—	—	149.4	149.6
	5′	6.20 *do*	115.2	6.73 *do*	115.7	6.81 *do*	6.88 *do*	115.8	116.0
	6′	6.43 *ddom*	117.8	6.98 *ddom*	118.7	7.21 *ddom*	7.37 *ddom*	118.4	118.6

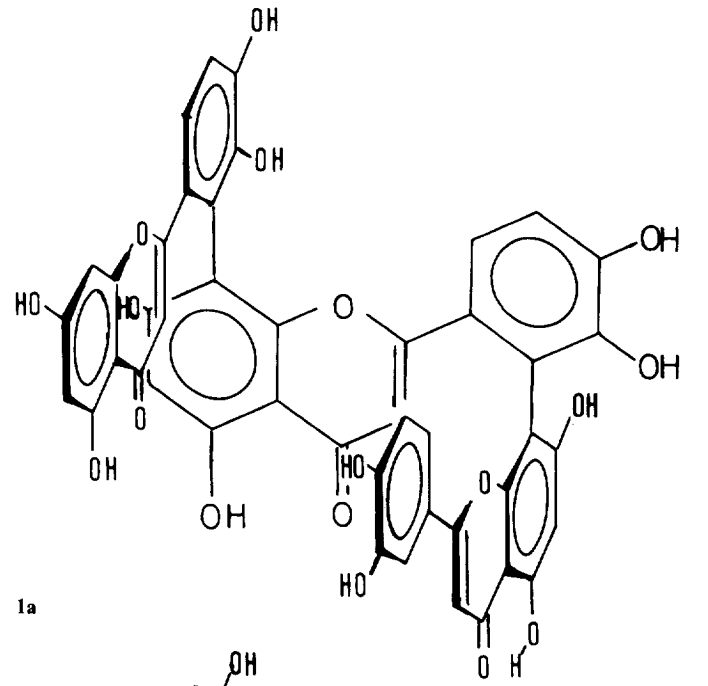
*All spectra were recorded in DMSO-d_6 at ambient temp. The frequencies of the instruments are given at the top of each spectrum. The ^1H signals were assigned on the basis of inverse detected ^{13}C–^1H correlations, H–H COSY, NOESY and/or ROESY spectra. With the NOESY spectra (200 MHz) of **1a** and **1b** and the ROESY spectra (500 MHz) of **1a** and **2** strong NOE interactions between H-3 and H-6′ of the subunits I and H-3 and H-2′ + 6′ of the subunits III were important for assigning the H-3 signals to the respective subunits. The signals of the quarternary carbon atoms of **1a** were assigned by means of a long-range ^{13}C–^1H correlation. The corresponding signals of **1b** and **2** were assigned by best fit; therefore, signals with almost identical chemical shifts may be interchangable.

†In ref. [3] the ^1H-signals of **1a** were assigned only on the basis of their chemical shift and multiplicity; the more sophisticated methods used in the present communication revealed that some assignements had to be changed. The spectra themselves are, however, identical within the limits of experimental error.

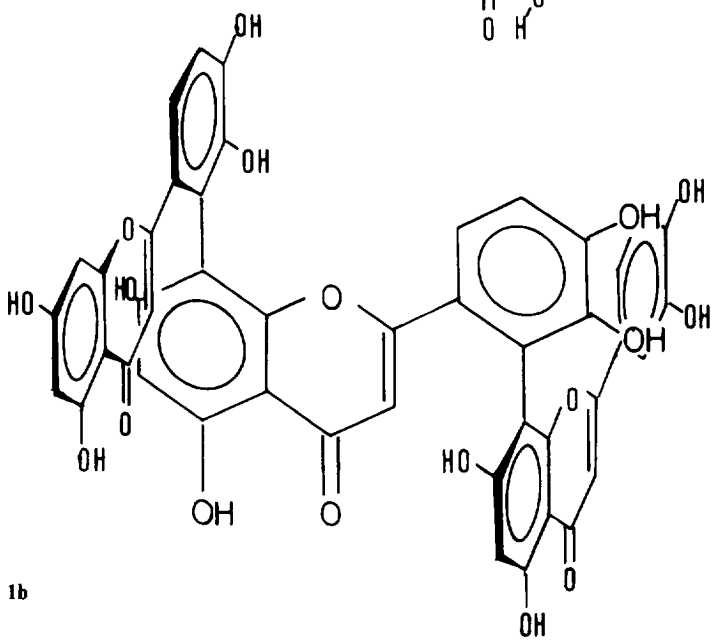


1a, b

Fig. 1.



1a



1b

Fig. 2.

IA- and IIIB-rings on the same side of the luteolin moiety II within each others shielding zone, must represent bartramiatriluteolin (**1a**), the ^{1}H NMR spectrum of which exhibits, compared with the spectrum of **1b**, extraordinarily strong shielded IA and IIIB protons (Table 1) and in consequence formula **1b** represents epibartramiatriluteolin (**1b**). In contrast to the strong mutual shielding between the IA- and IIIB-rings of **1a** no NOE interaction between the protons could be observed in the ROESY spectrum of **1a**, because the distance between the respective protons is too long for such an interaction.

The mixture that was left after removing **1b** consisted, according to its ^{1}H NMR spectrum, of **1a** and two further triluteolins. TLC (cellulose/40% acetic acid) of this mixture gave at ambient temperature only one spot; at 5°, however, it yielded two spots of $hR_f = 43$ and $hR_f = 55$. Therefore, it was subjected at this low temperature to column chromatography on Sephadex LH-20 (see Experimental). This resulted in four fractions. The first fraction contained a substance with $hR_f = 55$, which was identified by NMR as **1a**; then came a mixed fraction showing spots at $hR_f = 55$ and 43. The third fraction gave only one spot at $hR_f = 43$. The fourth fraction which followed the preceeding without any overlapping showed a spot with hR_f of again 55. These last two fractions were worked up and their NMR spectra were recorded. These spectra were to our disappointment identical and showed the signals of two triluteolins in a ratio of *ca* 3:4. TLC of the substances recovered from the NMR tubes were also identical and presented two spots at $hR_f = 55$ and 43. This indicates that one is dealing with two interconverting isomers; the rate of interconversion being at 5° slow enough to allow a chromatographic separation, but at ambient temperature fast enough for equilibration within hours, but still slow as compared with the NMR time-scale. Thus, the spectra of the mixture had to be analysed.

The LAMMA mass spectrum exhibited, as expected for a triluteolin, a [M − 1] anion at 853 *m/z*. From the ^{1}H NMR spectrum the data for the two components could be extracted on the basis of the integrals of the individual signals and with the aid of H–H and H–C correlations. The coupling pattern of the two components' protons is the same as with **1a** and **1b**; this identifies the BI2′ and BII2′ positions as bridgeheads. A comparison of the chemical shifts of the terminal B-rings of the biluteolins philonotisflavone and dicranolomin [4] suggests that the luteolin moieties III are linked via the IIIA6 positions and that the carbon signals at 93.1 and 93.7 ppm, which are both linked to uncoupled protons, must be assigned to the carbon atoms IIIA8. In consequence the two overlapping carbon signals at 98.2 ppm, correlated with the proton singlets at 6.16 and 6.20 ppm, must represent the carbon atoms IIA6; therefore, the second bridgehead of the luteolin moiety II must be at IIA8. In combination with the chemical shifts of the bridgehead carbon atoms (*v. supra* with **1a** and **1b**) these facts lead to IB2′ → 8IIA, IIB2′ → 6AIII-triluteolin as the structure of compound **2** with its two rotamers. This compound shall be named strictatriluteolin (**2**) after the specific epithet of *B. stricta*. In Figs 3 and 4, respectively, the structure of **2** is shown, along with its two equilibrating diastereomers **2a** and **2b**. The correlation between these two stereoisomers and the two sets of NMR spectra, extracted from the spectra of the mixture, is not as straightforward as in the case of **1a** and **1b**, but, since with **2b** the rings IA and IIIB are somewhat closer to each other than with **2a**, one might argue that **2b** represents the major component, whose IIIB-ring protons are somewhat more shielded than those of **2a**. This question, however, is not germane, since **2a** and **2b** are only two interconverting forms of strictatriluteolin **2**.

The finding of the triflavones **1a**, **1b** and **2**, along with biflavones having the same type of interflavonyl linkages

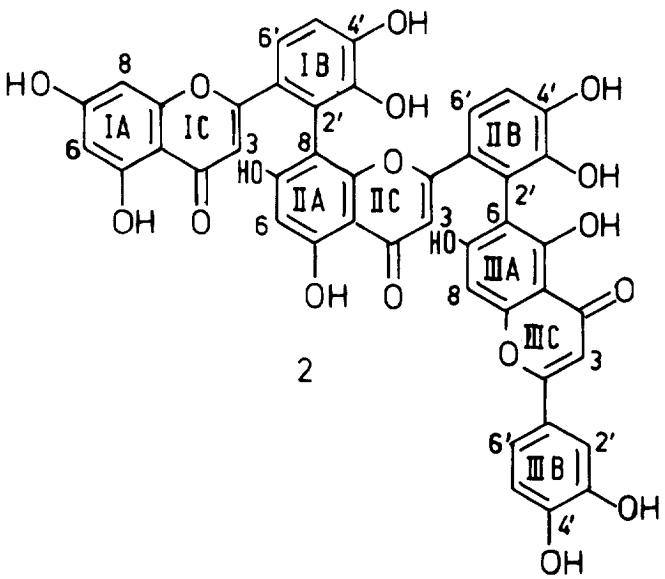


Fig. 3.

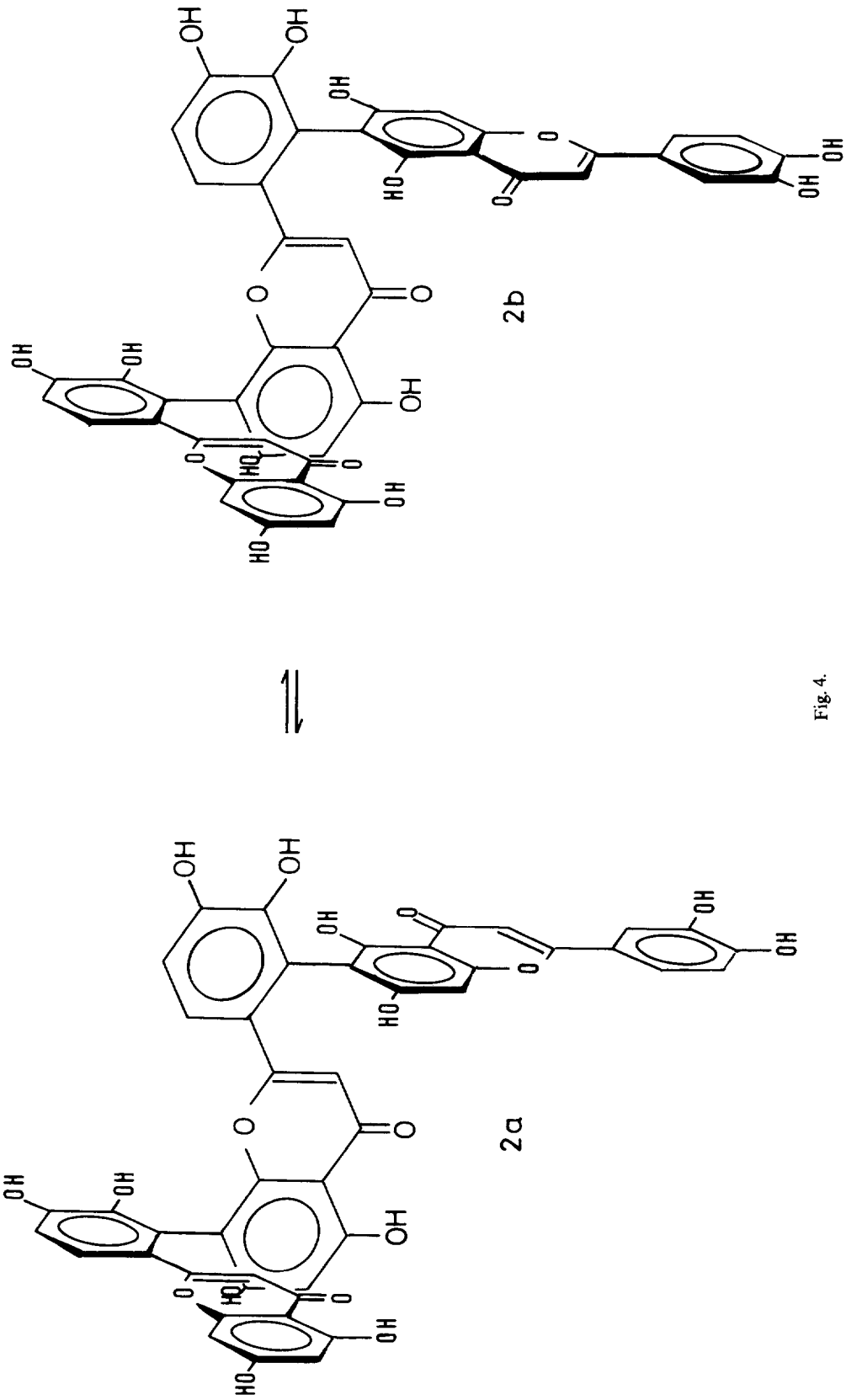


Fig. 4.

in the same moss species [1, 3], poses the question whether they are just a peculiar feature of the two *Bartramia* species, or whether they are the first members of a series of homologous oligoflavonoids that might constitute the phenolic encrusting material of the cellwalls of many mosses, a possibility which is discussed in ref. [13].

EXPERIMENTAL

The triflavone mixt. (100 mg), obtained as previously described [1], was fractionated as follows: by CC on polyamide-6 with Me_2CO–H_2O (4:1), which was monitored by TLC [micropolyamide and Me_2CO–HOAc–H_2O (2:1:1)], the mixt. was sepd into two frs. The first one (hR_f = 30) contained **1a** and **2a,b** whereas the second one consisted of pure **1b** (hR_f = 23). The mixt. of **1a** and **2a,b** was subjected to CC at 5° on Sephadex LH-20 with MeOH–H_2O (4:1) and the sepn monitored by TLC on cellulose with HOAc–H_2O (2:3) at 5°. The results of this sepn are described in Results and Discussion.

The yields were 20 mg **1a**, 10 mg **1b**, 20 mg **2** and 40 mg of a mixt. of **1a** and **2**. The matrix for the LAMMA-MS was nicotinamide.

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