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Epoxymurins A and B, Two Biogenetic Precursors of Annonaceous Acetogenins from *Annona Muricata*

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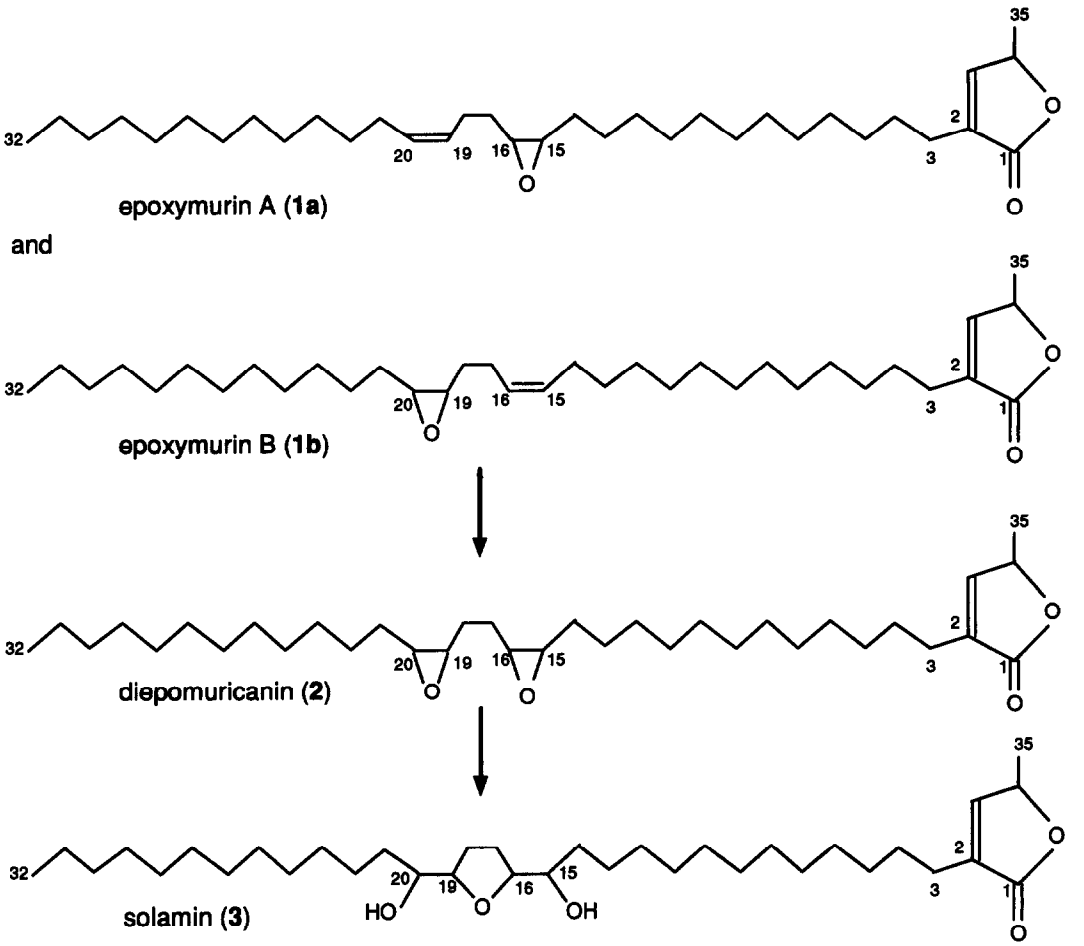
Abstract. NMR and mass spectroscopic methods have been used for the structure elucidation of two new Annonaceous acetogenin precursor compounds, epoxymurins A and B, isolated from the hexane extract of the stem bark of *Annona muricata*, together with diepomuricanin and solamin. Epoxymurins A and B provide an important missing link in the proposed biogenetic pathway for acetogenins containing one tetrahydrofuran ring.

Annonaceous acetogenins are a relatively new class of natural compounds with antitumoral, pesticidal and other bioactivities[1]. Recent investigations on the seeds of *Annona muricata* from French Guyana yielded several Annonaceous acetogenins containing one tetrahydrofuran (THF) ring[2-4]. As part of our ongoing studies on Annonaceous acetogenins, we have investigated the stem bark of *Annona muricata* growing in Kerala, India, and have isolated several acetogenins. In this paper we report the isolation and structure elucidation of two new compounds, named epoxymurins A and B (**1a** and **1b**), which provide an important missing link in the proposed biogenetic pathway for mono THF-containing acetogenins[1] and two known compounds, diepomuricanin (**2**) and solamin (**3**). The mixture of the isomers **1a** and **1b** has been isolated as an apparently pure compound (TLC, NMR). The structure elucidation of compounds **1a** and **1b**, **2** and **3** has been based on NMR (including 2-dimensional NMR) studies, and FAB tandem mass spectrometry of the lithium cationised complexes of **1a** and **1b**. Epoxymurins A and B belong to a structural subclass of Annonaceous acetogenins constituted only recently, the non-THF Annonaceous acetogenins, with only 4 members known as yet[1].

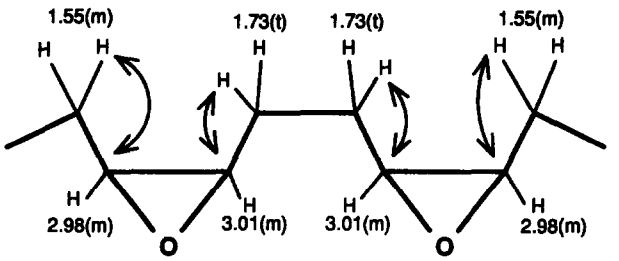
Chromatographic fractionation of the hexane extract of the stem bark of *Annona muricata* yielded several acetogenins. Compounds **1a** and **1b**, **2** and **3** were eluted in order of increasing polarity from the benzene-chloroform fractions. **3** has been identified as solamin by ^{1}H and ^{13}C NMR spectroscopy (see tables 1 and 2, respectively), electron impact (EI) and fast atom bombardment (FAB) mass spectrometry. Solamin was recently reported from the seeds of *Annona muricata*[3].



Scheme 1. Biogenetic pathway proposed for mono-THF containing acetogenins.

EI MS of **2** shows a molecular ion at *m/z* 546, whereas FAB MS reveals a $(M + H)^+$ ion at *m/z* 547. The consecutive loss of two molecules of H_2O yields peaks at *m/z* 529 and 511 in FAB MS. ^1H and ^{13}C NMR show the presence of a terminal γ-lactone moiety and a long aliphatic chain. No carbinol methine protons or carbons are present indicating the absence of hydroxyl groups. Similarly, no signals typical for THF rings are present. ^1H NMR of **2** (see table 1) shows four epoxymethine protons as a multiplet around δ 3.0 indicating the presence of two epoxide groups. This has been confirmed by a ^{13}C NMR-APT (attached proton test) spectrum (see table 2) showing two intense signals at δ 57.23 and 56.34. These signals are attributed to two epoxide groups with identical stereochemistry, which can be established as *cis* by general chemical shift considerations[5] and by comparison with the NMR spectral data of epoxyrollins A and B containing one epoxide group[7]. A ^1H-^1H COSY spectrum reveals that both epoxide groups are separated by two methylene units, yielding structural subunit A (chemical shifts, multiplicities, and correlations are indicated in Scheme 2).

EI and FAB MS do not give any confirmatory information on the location of the epoxide groups in the aliphatic chain. FAB MS of lithium complexed molecules was recently proposed as a technique to localize



Scheme 2. Structural subunit A, assigned on the basis of ^1H-^1H COSY NMR.

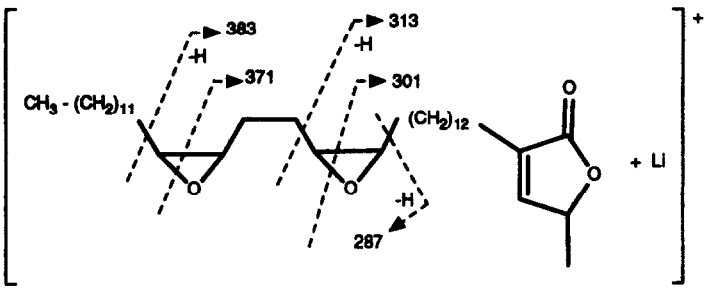
epoxide groups in long aliphatic chains[4,6]. Annonaceous acetogenins were reported to form stable complexes with Li cations during FAB, and their collision-induced dissociation was found to yield structurally useful information. The product ion spectrum of $(M + Li)^+$ ions (*m/z* 553) of **2** shows that the epoxide groups can be located between C-15 and C-16, and between C-19 and C-20. Thus, **2** can be identified as diepomuricanin, a compound reported recently[4].

Table 1. ^1H NMR Data of Compounds **1-2** (400 MHz, $CDCl_3$) and **3** (200 MHz, $CDCl_3$)

Hydrogen no.	**1a**	**1b**	**2**	**3**
3	2.26 t (7.4)	2.26 t (7.4)	2.30 t (7.8)	2.33 t (7.3)
4	1.5-1.6 m	1.5-1.6 m	1.5-1.6 m	1.5 m
5-13	1.26 br	1.26 br	1.26 br	1.26 br
14	1.5-1.6 m	2.04 q (6.7)	1.5-1.6 m	1.5 m
15	2.92 m	5.40 m	2.98 m	3.41 m
16	2.92 m	5.40 m	3.01 m	3.82 m
17	1.5-1.6 m	2.21 m	1.73 t (5.2)	2.00 m
18	2.21 m	1.5-1.6 m	1.73 t (5.2)	2.00 m
19	5.40 m	2.92 m	3.01 m	3.82 m
20	5.40 m	2.92 m	2.98 m	3.41 m
21	2.04 q (6.7)	1.5-1.6 m	1.5-1.6 m	1.5 m
22-31	1.26 br	1.26 br	1.26 br	1.26 br
32	0.88 t (6.8)	0.88 t (6.8)	0.92 t (6.8)	0.88 t (6.8)
33	6.98 d (1.7)	6.98 d (1.7)	7.01 d (1.8)	7.00 d (1.7)
34	4.98 dq (6.9/1.7)	4.98 dq (6.9/1.7)	5.02 dq (6.9/1.8)	5.00 dq (6.8/1.7)
35	1.40 d (6.9)	1.40 d (6.9)	1.44 d (6.7)	1.40 d (6.8)

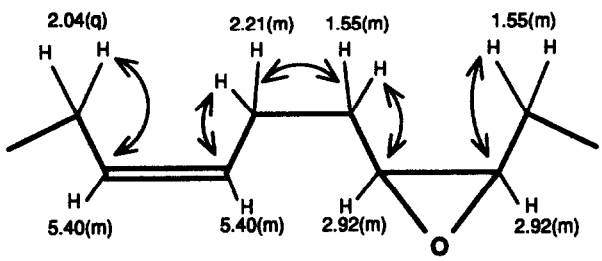
The product ion spectrum of the $(M + Li)^+$ ions at *m/z* 553 is very similar to the spectrum obtained for

diepomuricanin (2) by Laprévote *et al.*[4]. Product ions are detected at *m/z* 535, 525 and 509, which are due to the loss of H_2O, CO and CO_2, respectively, of which the losses of CO and CO_2 appear to be characteristic for the γ-lactone ring. Diagnostic product ions which enable to localize the two epoxy functions are present at *m/z* 301, 313, 371 and 383. The presence of product ions at *m/z* 287 is also consistent with the proposed structure. A rationalization of these structurally informative product ions is given in Scheme 3. In contrast to the product ion spectrum obtained by Laprévote *et al.*[4], the spectrum obtained under the experimental conditions used in this study, is more complex. This is probably due to the use of more energetic collisions, which give rise to remote charge fragmentations involving 1,4-hydrogen eliminations (for reviews, see references 8 and 9) and to further fragmentation of diagnostic product ions. For example, the three abundant diagnostic ions at *m/z* 383, 371 and 313 show corresponding ions at *m/z* 355, 343 and 285, respectively, which can be rationalized by an additional loss of CO.



Scheme 3. Structurally informative product ions formed by collision-induced dissociation of $(M + Li)^+$ ions of diepomuricanin (2).

Compounds **1a** and **1b**, which we named epoxymurins A and B, show a molecular ion at *m/z* 530 in EI MS, and a $(M + H)^+$ ion at *m/z* 531 in FAB MS. The loss of one molecule of H_2O yields peaks at *m/z* 512 in EI MS and *m/z* 513 in FAB MS. FAB MS of lithium complexed **1a** and **1b** shows a $(M + Li)^+$ ion at *m/z* 537. ^1H and ^{13}C NMR (APT) (see tables 1 and 2, respectively) indicate the presence of a terminal γ-lactone moiety and a long aliphatic chain. No hydroxyl groups or THF rings appear to be present. In contrast to **2**, a two proton multiplet at δ 2.92 in ^1H NMR, and two epoxy methine carbons at δ 57.23 and δ 56.70 in ^{13}C NMR, indicate the presence of only one epoxide group. The presence of a disubstituted double bond is evident from a two proton multiplet at δ 5.40 in ^1H NMR, and two CH signals at δ 128.18 and 130.87 in ^{13}C NMR. As for compound **2**, the stereochemistry of the epoxide group has been found to be *cis*. Comparison

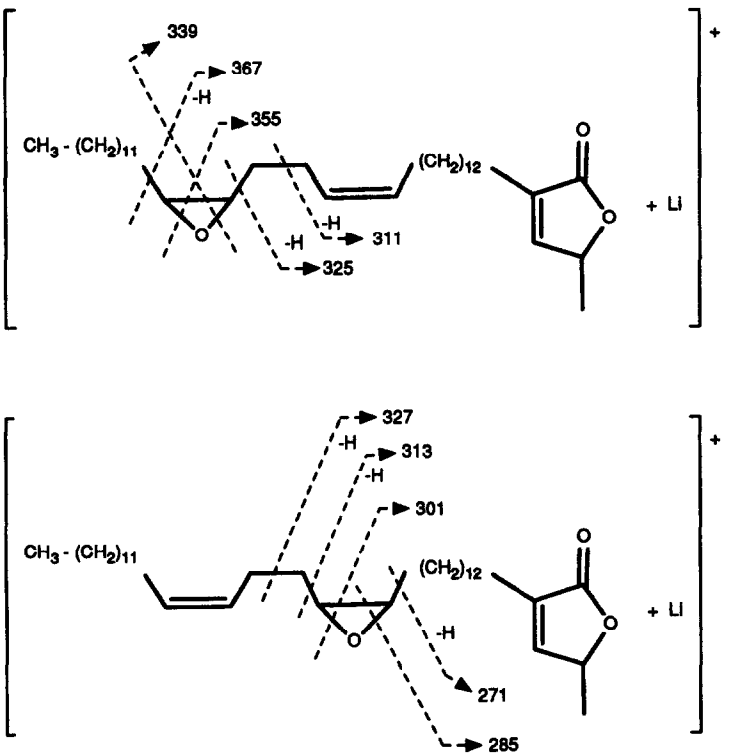


Scheme 4. Structural subunit B, assigned on the basis of ^1H-^1H COSY NMR.

of the ^{13}C NMR chemical shifts of the carbon atoms α to the double bond with those of *cis* and *trans* alkenes[10] also points to a *cis* geometry for the double bond. Examination of the ^{1}H-^{1}H COSY spectrum reveals that the double bond and the epoxide moiety are separated by two methylene units, thus yielding structural subunit B (Scheme 4).

The location of structural subunit B in the aliphatic chain has been based upon a detailed FAB MS/MS study of the lithium complex, which shows that fraction **1** is a mixture of two isomers, with the epoxy group located between C-15/C-16 and the double bond between C-19/C-20 (**1a**, epoxymurin A), or *vice versa* (**1b**, epoxymurin B). The product ion spectrum of $(M + Li)^+$ ions of fraction 1 is given in Figure 1. Diagnostic product ions, which enable to localize the epoxy function, are observed at *m/z* 339, 325, 367, 355, 311, 327, 313, 301, 285 and 271, and are consistent with the presence of two geometric isomers, each containing one epoxy function and one double bond. A rationalization of these ions is summarized in Scheme 5. It can also be noted that a lot of these structurally informative product ions show corresponding ions due to additional loss of 2 u (H_2). Unfortunately, we could not find diagnostic product ions, which enable to determine the double bond position.

As in the case of diepomuricanin (**2**), the product ion spectrum of $(M + Li)^+$ ions of fraction **1** also show ions due to the loss of H_2O (*m/z* 519), CO (*m/z* 509) and CO_2 (*m/z* 493) and a series of ions formed by remote charge fragmentations in the aliphatic chain.



Scheme 5. Structurally informative product ions formed by collision-induced dissociation of $(M + Li)^+$ ions of the two isomeric epoxymurins A and B (**1a** and **1b**) present in fraction **1**.

These results are in agreement with the hypothetical biosynthetic pathway of mono THF-containing acetogenins, which involves the epoxidation of a *cis*-diene followed by ring openings of the epoxide moieties and ring closure to form a tetrahydrofuran ring[1]. The isolation of epoxymurins A and B (**1a** and **1b**), diepomuricanin (**2**) and solamin (**3**) as a biogenetic series from the stem bark of *Annona muricata* provides an important argument in favour of this hypothesis.

Table 2. ^{13}C NMR Data of Compounds **1-3** (50 MHz, $CDCl_3$)

Carbon no.	1a	1b	2	3
1	173.85	173.85	173.73	173.85
2	134.10	134.10	134.20	134.20
3	25.05	25.05	25.05	25.05
4	27.28	27.28	27.27	27.33
5-12	29.2-29.6	29.2-29.6	29.2-29.6	29.2-29.6
13	26.51 *	26.51 *	26.51 *	25.46 *
14	24.18 *	24.18 *	24.88 *	33.87
15	57.23 #	130.87 #	57.23 #	74.28
16	56.70 #	128.18 #	56.34 #	82.52
17	27.86 *	27.86 *	27.68 *	28.03
18	27.74 *	27.74 *	27.68 *	28.03
19	128.18 +	56.70 +	56.34 #	82.52
20	130.87 +	57.23 +	57.23 #	74.05
21	27.10 *	27.10 *	26.51 *	33.29
22	25.05 *	25.05 *	25.05 *	25.05 *
23-29	29.2-29.6	29.2-29.6	29.2-29.6	29.2-29.6
30	31.83	31.83	31.83	31.83
31	22.60	22.60	22.60	22.60
32	14.01	14.01	14.01	14.01
33	148.74	148.74	148.74	148.80
34	77.32	77.32	77.26	77.32
35	19.10	19.10	19.10	19.10

*, #, + assignments may be interchanged within the same column

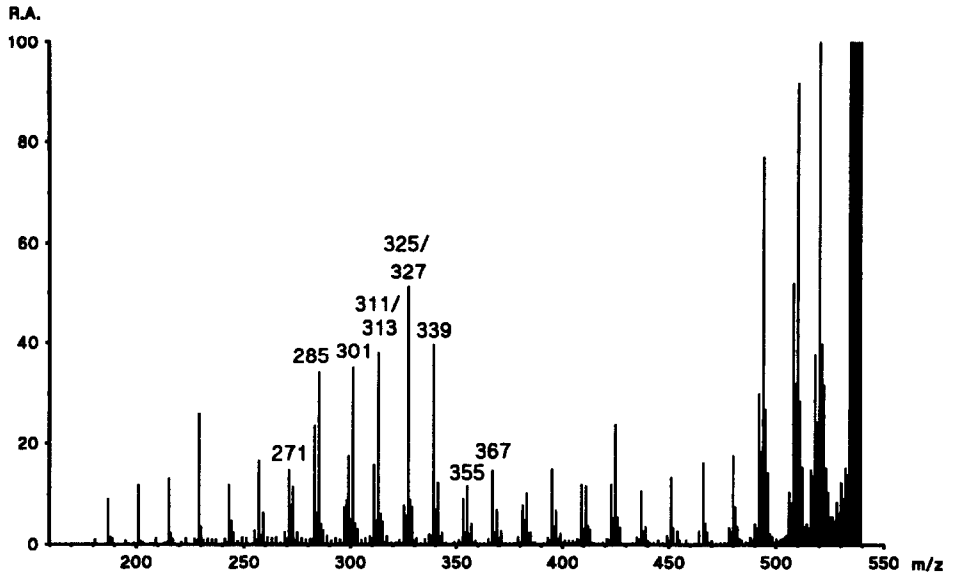


Fig. 1. Product ion spectrum of $(M + Li)^+$ ions (*m/z* 537) of fraction **1** containing a mixture of epoxymurin A and B (**1a** and **1b**). Only product ions yielding information on the location of the epoxy function are indicated (see also Scheme 5). Other product ions are present at *m/z* 519, 517, 509, 507, 493, 491, 479, 465, 451, 437, 425, 423, 411, 409, 397, 395, 383, 381, 369, 353, 341, 299, 283, 273, 257, 243, 229, 215, 201 and 187.

EXPERIMENTAL

Stem bark of *Annona muricata* was collected in Andoopara, Paravur, Quilon, Kerala, India. Powdered stem bark (1 kg) was extracted with n-hexane at room temperature. Evaporation of the n-hexane extract under reduced pressure yielded 10 g of an oily residue, which was chromatographed on a silica gel column with n-hexane, benzene and $CHCl_3$. Elution was monitored by TLC on silica gel. Spots were detected by spraying with vanillin/sulphuric acid reagent followed by heating at 110°C for a few minutes. Compounds **1a** and **1b** (as a mixture) and **2** were isolated from benzene-$CHCl_3$ fractions, and **3** from $CHCl_3$ fractions. Purification was performed by preparative TLC on silica gel. Mixture **1a-b** was found to undergo mild aerial oxidation to form **2** in small amounts when kept at room temperature for several days. The yields of **1**, **2** and **3** were 50, 75 and 100 mg, respectively.

One-dimensional ^1H NMR spectra of **1a-b** and **2**, and all two-dimensional NMR spectra were recorded on a 400 MHz Varian Unity instrument using Varian software and standard recording conditions. The ^1H NMR spectrum of **3** and all ^{13}C NMR spectra were recorded on a 200 MHz Jeol FX-200 instrument. ^{13}C NMR-APT (attached proton test) spectra were obtained using a delay time of 7 ms. All NMR spectra were recorded at room temperature in $CDCl_3$ with TMS as an internal standard (δ-scale).

Mass spectra were recorded on a hybrid mass spectrometer (VG70SEQ; EBqQ configuration) equipped with a FAB saddle field atom gun. For FAB, xenon atoms of 8 keV with a beam flux of 1 mA were used. m-Nitrobenzylalcohol was used as a liquid matrix. Lithiation experiments were performed using m-nitrobenzylalcohol saturated with LiI. Product ion spectra were obtained by subjecting $(M + Li)^+$ ions to high

energy (8 keV) collisions with helium in the first field-free region of the instrument. The precursor ion beam was reduced to approximately 50% of its original value. Product ion scans were performed by B/E linked scanning and spectra were obtained by accumulating 10 scans.

Epoxymurin (1): Wax; EI MS: *m/z* (relative intensity, %) 530 ($M^{+\cdot}$, 10) ; FAB MS: *m/z* 531 $(M + H)^+$; 537 $(M + Li)^+$; product ion spectrum $(M + Li)^+$: Fig. 1; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Diepomuricanin (2): Wax; EI MS: 546 (M^+, 5); FAB MS: 547 $(M + H)^+$; 553 $(M + Li)^+$; product ion spectrum $(M + Li)^+$: 535 (40), 533 (20), 525 (83), 523 (42), 509 (100), 507 (50), 495 (12), 481 (16), 467 (12), 453 (14), 441 (24), 439 (15), 427 (16), 425 (12), 399 (15), 397 (12), 385 (15), 383 (48), 371 (37), 355 (18), 343 (21), 329 (19), 327 (25), 315 (18), 313 (73), 311 (12), 301 (28), 299 (14), 287 (27), 275 (31), 273 (18); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Solamin (3): Wax; EI MS: 528 [$M^{+\cdot}$-(2xH_2O), 8], 347 (43), 319 (28), 295 (100), 267 (76); FAB MS: 565 $(M + H)^+$; 571 $(M + Li)^+$; ^1H NMR: Table 1; ^{13}C NMR: Table 2; trimethylsilylether; EI MS: 618 ($M^{+\cdot}$-TMSOH, 5), 455 (31), 437 (52), 409 (31), 367 (100), 339 (71), 271 (42).

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