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STRUCTURAL ELUCIDATION OF TWO NEW ACETOGENINS, EPOXYROLLINS A AND B, BY TANDEM MASS SPECTROMETRY

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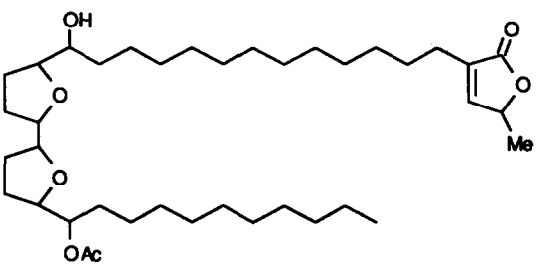
Abstract: Tandem mass spectrometry has been used to elucidate the structure of γ-lactonic acetogenins, epoxyrollins A and B, which are the first examples of acetogenins having an epoxide in place of tetrahydrofuran moiety.

Since the isolation, in 1982, of uvaricin (1) from *Uvaria accuminata*,[1] fifteen mono- or bistetrahydrofuranic acetogenins have been isolated from other Annonaceae, most of them exhibiting antitumoral, cytotoxic, antibacterial or pesticidal properties.[2]



(1)

As part of our continuing investigation of the chemistry of this family, we have isolated fifteen acetogenins from the petroleum ether leaf extract of *Rollinia ulei* Diels.[3] Two of these, the epoxyrollins A (2) and B (3), present a novel structure in which the bistetrahydrofuran system is replaced by an epoxy group. The mixture of these two compounds was isolated as an apparently pure compound (TLC, NMR).

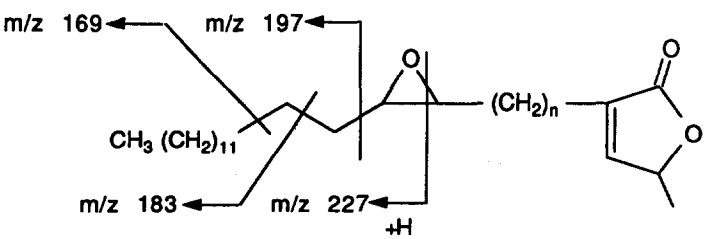
The strong absorption at 1750 cm^{-1} in the IR spectrum and the positive reaction with Kedde's reagent suggest the presence of an α,β-unsaturated γ-lactone, which is confirmed by comparison of the ^1H NMR data with those of previously isolated acetogenins.[2] Along with the signals corresponding to the γ-lactone moiety, the ^1H NMR spectrum shows characteristic chemical shifts for a long hydrocarbon chain (1.26 ppm) with a terminal methyl group (0.88 ppm) and a system of two protons at 2.92 and 2.98 ppm which can be attributed to a cis-disubstituted epoxide (J 4.5 Hz). In addition, the heterocorrelated ^1H-^{13}C NMR spectrum shows the corresponding oxygen-bearing carbons of the epoxide at 56.7 and 57. 8 ppm.[4]

To localize the epoxide on the aliphatic chain, periodic acid cleavage was carried out. Surprisingly, this reaction yielded a complex mixture of unidentifiable products suggesting that the "compound" under investigation was not really pure. This hypothesis was confirmed by mass spectrometry. Under positive ions desorption chemical ionisation (DCI) conditions, the DCI/NH_4^+ mass spectrum displays two intense peaks corresponding to the ammonium adduct ions of two compounds: epoxyrollin A ($[M_A+NH_4]^+$ at m/z 592) and epoxyrollin B ($[M_B+NH_4]^+$ at m/z 564). Two other peaks of weak intensity are attributed to the protonated molecules (M_AH^+ at m/z 575 and M_BH^+ at m/z 547). The presence of two unseparable homologues in the mixture was thus indicated, but the very weak abundances of fragment ions did not allow any further interpretation of the DCI mass spectrum.

The separation of the mixture by HPLC or GC was unsuccessful. For this reason, tandem mass spectrometry experiments (MS/MS) were performed by using low energy collisionnally activated dissociation (CAD), allowing a selective analysis of each of the two molecular species introduced as a mixture in the CI source.

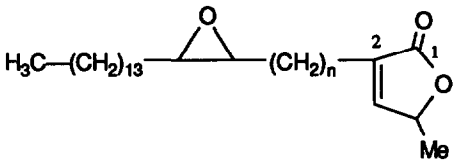
Under positive ion conditions, the CAD spectra of the adduct ions $[M_A+NH_4]^+$ and $[M_B+NH_4]^+$ display in each case a loss of NH_3 followed by successive losses of two molecules of H_2O. The absence of significant diagnostic daughter ions, even at higher energy levels (E_{lab}:15, 25 or 35 eV, multiple collision conditions), did not help to localize the epoxide. In the negative mode, the DCI/NH_2^- mass spectrum of the mixture shows the deprotonated molecules of epoxyrollins A and B ($[M_A-H]^-$ at m/z 573 and $[M_B-H]^-$ at m/z 545), together with the ions resulting from the electron capture $M_A^{-\cdot}$ at m/z 574 and $M_B^{-\cdot}$ at m/z 546.

The CAD spectra of the deprotonated molecules $[M_A-H]^-$ and $[M_B-H]^-$ present very similar fingerprints. Indeed, the two CAD spectra show common daughter ions at m/z 227, 197, 183, and 169. The m/z 227 fragment ion results from a ring cleavage of the epoxy-site with a H transfer, and contains the hydrocarbon chain with the terminal methyl group.[5] The daughter ions at m/z 197, 183, and 169 are attributed to C-C bond cleavages in the α, β, or γ positions with regard to the epoxide group towards the terminal methyl (Scheme 1). Other fragment ions at m/z 209 and 181 result from a successive loss of H_2O and C_2H_4 from the m/z 227 ion.



Scheme 1

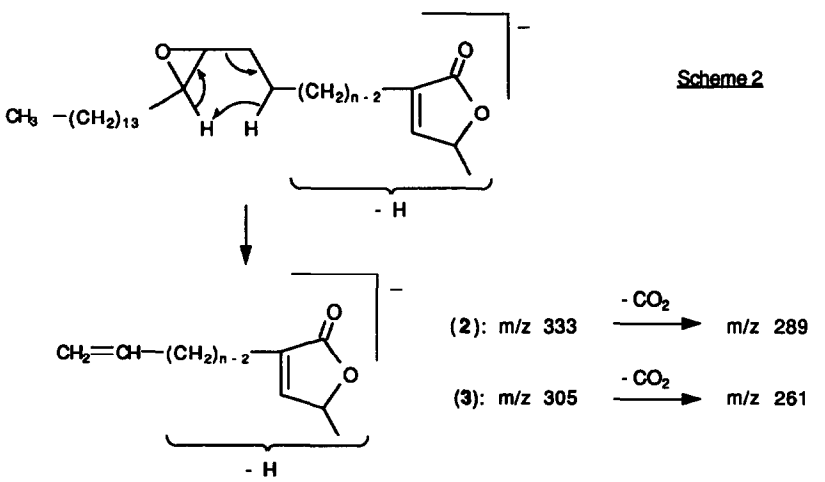
These results locate the epoxide group in the molecules with respect to the methyl group at an identical position. Thus, structural difference of epoxyrollin A and B is localized between the epoxide and the lactone rings. Consequently, epoxyrollin A and B can be assigned structures (2) and (3), respectively :



(2) n = 17
(3) n = 15

All the daughter ions of the deprotonated epoxyrollin A containing the lactone moiety appear 28 Da higher than the corresponding daughter ions of epoxyrollin B. Furthermore, the loss of CO_2 from most of these daughter ions is a diagnostic of the presence of the lactone ring

The deprotonation of the molecules on the epoxide or lactone sites leads to the formation of particularly stable ions. The charge stability on the lactone site could explain the formation of the daughter ions at m/z 333 (epoxyrollin A) and m/z 305 (epoxyrollin B) by a remote charge type process as shown in Scheme 2, by analogy with the mechanism proposed by Gross *et al.*[6] This is somewhat surprising in view of previous works which demonstrated that the yield of such process induced under low collision energy conditions was very weak.[7] Indeed, remote charge type fragmentations have been described earlier in CAD conditions on fatty acid derivatives at 8 KeV collision energy.[6]



Scheme 2

Other cleavages of epoxyrollins between the C2-C3 and C3-C4 positions involving elimination of neutrals lead to a loss of 100 or 114 Daltons from the parent $[M - H]^-$ ion of each homologue. The CAD spectra display the two corresponding ion peaks at m/z 473 and 459 for epoxyrollin A and m/z 445 and 431 for epoxyrollin B.

In conclusion, the use of tandem mass spectrometry was essential for the structural determination of an unresolved mixture of epoxyrollin A and B. This analytical method, because of the excellent reproducibility of CAD spectra, gives very reliable information for structural analysis. The CAD spectra exhibit structurally significant fragment ion peaks which allow suitable comparison between different homologues or isomers.

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References and notes

1) S. D. Jolad, J. J. Hoffmann, K. H. Schram, J. R. Cole, M. S. Tempesta, G. R. Kriek, and R. B. Bates, *J. Org. Chem.*, 1982, **47**, 3151.

2) Y.-H. Hui, J. K. Rupprecht, Y. M. Liu, J. E. Anderson, D. L. Smith, C.-J. Chang, and J. L. McLaughlin, *J. Nat. Prod.*, 1989, **52**, 463 [and ref. cited herein].

3) *R. ulei* (Diels), was collected in Peru, in November, 1984, and identified by Pr. P. J. M. Maas. Voucher specimens (Maas, 5972) are deposited in the herbaria of the Rijksuniversiteit, Utrecht.

4) The mass spectra and CAD spectra were recorded on triple quadrupole R-30-10 (Nermag) under conventional conditions using Argon as collision gas.
Epoxyrollin A **2**: (in admixture with epoxyrollin B) $C_{38}H_{70}O_3$; IR, $\nu_{max.}$ (film) 1750 cm^{-1}; MS (positive desorption chemical ionization by ammonia), m/z 592 ($[M+NH_4]^+$) (100 %), 575 (MH^+) (2); (negative desorption chemical ionization by ammonia) m/z 574 ($M^{-\cdot}$) (100 %), m/z 573 ($[M-H]^-$) (46); (CAD spectrum of the m/z 573 ion, 20 eV collision energy) m/z 529 (34 %), 511 (100), 499 (12), 473 (11), 459 (17), 375 (6), 359 (16), 333 (7), 331 (69), 313 (48), 305 (25), 289 (72), 277 (46), 227 (41), 209 (40), 197 (13), 183 (4), 181 (5), 169 (13); ^1H NMR ($CDCl_3$, 250 MHz), δ ppm 0.88 (3H, t, *J* 6.5, 35-Me), 1.26 (54H, m, aliphatic CH_2), 1.35 (3H, d,*J* 7, 38-Me), 1.60 (4H, m, 19 and 22-CH_2), 2.26 (2H, tdd, *J* 7, 1.8, 1.5, 3-CH_2), 2.92 and 2.98 (2H, 2td, *J* 7, 4.5, 20 and 21-CH), 4.94 (1H, qtd, *J* 7, 1.8, 1.5, 37-CH), 6.95 (1H, td, *J* 1.5, 1.5, 36-CH); ^{13}C NMR ($CDCl_3$, 100 MHz), δ ppm 14.3 (C-35), 26.8-26.9 (aliphatic CH_2), 19.4 (C-38), 25.2 (C-19 and C-22), 25.4 (C-3), 56.7 and 57.8 (C-20 and C-21), 77.6 (C-37), 134.7 (C-2), 149.2 (C-36), 174.2 (C-1).
Epoxyrollin B **3**: (in admixture with epoxyrollin A) $C_{36}H_{66}O_3$; IR, $\nu_{max.}$ (film) 1750 cm^{-1}; MS (positive desorption chemical ionization by ammonia) m/z 564 ($[M+NH_4]^+$) (20 %), 547 (MH^+) (1); (negative desorption chemical ionization by ammonia) m/z 546 ($M^{-\cdot}$) (20 %), 545 ($[M-H]^-$) (58); (CAD spectrum of the m/z 545 ion, 20 eV collision energy) m/z 501 (21 %), 483 (100), 471 (7), 445 (4), 431 (17), 347 (3), 331 (16), 305 (9), 303 (39), 285 (23), 277 (20), 261 (38), 249 (27), 227 (16), 209 (27), 197 (44), 183 (4), 181 (7), 169 (16); the ^1H NMR ($CDCl_3$, 250 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data are identical to those of epoxyrollin A (**2**).

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6) N. J. Jensen and M. L. Gross, *Mass Spectrom. Reviews*, 1987, **6**, 497; *ibid*, 1988, **7**, 41; J. Adams, L. J. Deterdin, and M. L. Gross, *Spectros. Int. J.*, 1987, **5**, 199.

7) V. H. Wysocki, M. E. Bier and R. G. Cooks, *Org. Mass Spectrom.*, 1988, **23**, 627.

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