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COHIBINS A AND B, ACETOGENINS FROM ROOTS OF *ANNONA MURICATA**

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Key Word Index—*Annona muricata*; Annonaceae; roots; acetogenins; cohibins A and B.

Abstract—Cohibins A and B, biogenetic intermediates of acetogenins, were characterized using tandem mass spectrometry (MS/MS) from the roots of *Annona muricata*. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Annona muricata is a small tropical tree whose edible fruits are used commercially for the production of juice, candy and sherbets [1]. Intensive chemical investigations of the seeds and leaves of this species has led to the isolation of a great number of acetogenins characterized by a long aliphatic chain bearing a terminal methyl-substituted unsaturated γ-lactone and one or two tetrahydrofuran rings, sometimes replaced by epoxy rings and (or) double bonds [2]. These compounds display some interesting biological activities, such as cytotoxicity, antitumoral, antiparasitic and pesticidal properties.

Roots used in traditional medicine for antiparasitical and pesticidal properties [1] have not been studied for their acetogenin content. In the course of our search for key biogenetic intermediate of acetogenins, we have isolated, in addition to the known acetogenins, annonacin, epomuricenins-A and -B [3] and montecristin [4], two new products, cohibins A (**1a**) and B (**1b**), as an unresolvable mixture of isomeric acetogenins.

RESULTS AND DISCUSSION

The dried and powdered roots were extracted with methanol. The methanol extract, after concentration under vacuum, was partitioned between water and methylene chloride. The organic layer was dried and submitted to successive fractionations by column chromatography. Preparative HPLC led to the isolation of the cohibins (Fig. 1) as a white solid wax.

The M_r of the cohibins (548) was determined on the basis of a CI-mass spectrum which exhibited a $[M+H]^+$ at m/z 549. The high-resolution-CI-mass spectrum 549.4707 (calc. 549.4693 for $C_{35}H_{65}O_4$), indicated the molecular formula $C_{35}H_{64}O_4$ for both cohibins A and B. IR and UV spectra, along with a positive Kedde's reaction, revealed the presence of an α,β-unsatured γ-lactone, confirmed by ^{1}H NMR spectroscopy (Table 1) [2]. In addition, the ^{1}H and ^{13}C NMR spectra revealed by the absence of the corresponding characteristic chemical shifts [2], that cohibins possess neither THF rings nor epoxy groups. The existence of two vicinal hydroxyl groups was suggested by a two-proton multiplet at δ 3.42 [5] and two carbon resonances at δ 74.61 and δ 74.27. The presence of one isolated double bond was shown by multiple resonances due to two olefinic protons at δ 5.38–5.40 and by two carbon peaks at δ 128.97 and δ 131.07. Selective irradiations at 400 MHz of the α-methylenes (δ 2.05 or δ 2.20) provided a doublet for one of the olefinic protons; the resulting coupling constants of 11 Hz were consistent with a Z-geometry of the double bond. This stereochemistry was in agreement with the ^{13}C NMR chemical shifts of the α-methylene carbons at δ 23.40 and δ 27.20 [6].

The COSY spectrum showed correlation between δ 3.42 (H16), δ 1.55 (H17), δ 2.20 (H18) and δ 5.40 (H19), confirmed by ^{1}H–^{13}C correlations in the HMBC spectrum. This revealed that the double bond and vicinal diol moieties were separated by two methylene groups (Fig. 2), as in coriadienin [7], venezenin [8] or giganin [9] (three linear acetogenins isolated from the Annonaceae). These results are in agreement

* Part 51 in the series 'Acétogénines des Annonaceae'. For part 50 see A. I. Waechter, D. Fall, R. Hocquemiller, A. Laurens and A. Cavé, *Phytochemistry*, 1997, **44**, 1537.

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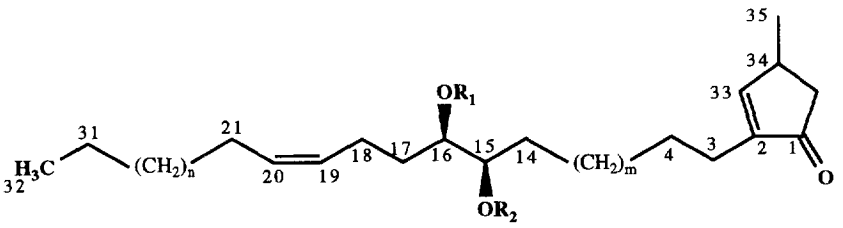


Cohibin A (1a); R_1=R_2=H; m=9; n=9 (numerotation).
Cohibin B (1b); R_1=R_2=H; m=7; n=11.
Cohibin acetonide (2) R_1,R_2=$C(CH_3)_2$.
Absolute configurations may be inverted.

Fig. 1. Structure of cohibins A and B.

Table 1. ^1H and ^{13}C NMR data ($CDCl_3$, δ) of cohibin A (**1a**)

Atom no	^1H NMR	^{13}C NMR
1	–	173.9
2	–	134.3
3	2.26 *m*	25.3
4	1.55 *m*	27.3
5	1.26 *m*	29.6
6/13	1.20–1.40	25.4–29.5
14	1.41 *m*	33.5
15	3.42 *m*	74.6*
16	3.42 *m*	74.3*
17	1.55 *m*	33.5
18	2.20 *m*	23.4
19	5.40 *m*	129.0
20	5.38 *m*	131.1
21	2.05 *m*	27.2
22/30	1.20–1.40	25.4–29.5
31	1.30 *m*	22.6
32	0.87 *t*	14.1
33	6.99 *d*	149.0
34	5.00 *dq*	77.5
35	1.42 *t*	19.0

* Interchangeables.

$J_{3\text{-}4}$ = 7.15 Hz; $J_{20\text{-}21}$ = 11 Hz; $J_{31\text{-}32}$ = 7.04 Hz; $J_{33\text{-}34}$ = 1.68 Hz; $J_{34\text{-}35}$ = 6.78 Hz.

with a partial structure but do not allow the location of the diol system and their relative position to the lactone ring or the terminal methyl. This structural problem was further solved by mass spectrometry.

The Cl-mass spectrum showed two successive losses of water. Taking into account the very few fragmentations in the El-mass spectrum (Fig. 3) of these linear acetogenins, their structures were assigned using tandem mass spectrometry. This spectrum displayed a number of fragment ion peaks, among which two different ion series could be distinguished, depending on whether they possessed the terminal lactone ring. The first ion series (series A), containing the lactone moiety, was formed by remote charge fragmentations [10] of the whole aliphatic chain leading to the typical pattern of successive ion peaks separated by 14 mu. This ion series was interrupted at substitution or unsaturation sites, thus allowing their location on the alkyl chain (Fig. 4) Fragment ion peaks at *m/z* 385 and *m/z* 345 were indicative of a double bond (C19–C20). Furthermore, the loss of water from the fragment ions at *m/z* 345 accounted for the location of the hydroxyl groups between the double bond and the lactone ring. Their vicinal C15 and C16 positions were established by the presence of three ion peaks separated by 30 mu (*m/z* 271, 301 and 331) (Fig. 4). A close study of the A series showed that fragment-ion peaks observed in the spectrum, shifted by 28 mu of weak intensity and proportionally connected with the main fragment peaks series (*m/z* 317 and *m/z* 357 for the double bond C17–C18; *m/z* 243, 273 and 303 for the diol system C13–C14, were assigned to the minor isomer cohibin B (**1b**). No other modifications were observed below *m/z* 243. It is also noteworthy that the low mass end of the A series consisted of a radical ion at *m/z* 118, formed by cleavage β to the lithiated lactone.

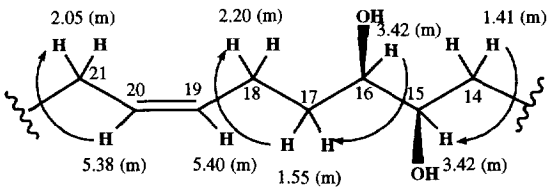


Fig. 2. Structural subunit, assigned on the basis of ^1H–^1H COSY NMR.

The B series corresponded to ions containing the methyl-terminal side-chain of the lithiated molecule. The loss of the lactone ring occurred by β-cleavage, with loss of *m/z* 112 from the precursor $[M+Li]^+$ ion (fragment-ion peak at *m/z* 443) [11]. the sequential remote charge-site fragmentations of the aliphatic chain between the lactone and the diol led to successive ion peaks in the range *m/z* 443-289 and 443-317 for **1a** and **1b**, respectively (Fig. 5).

In order to determine the relative configuration of the vicinal hydroxyl groups, the acetonide derivatives (**2**) were prepared [12]. The ^1H NMR spectrum of

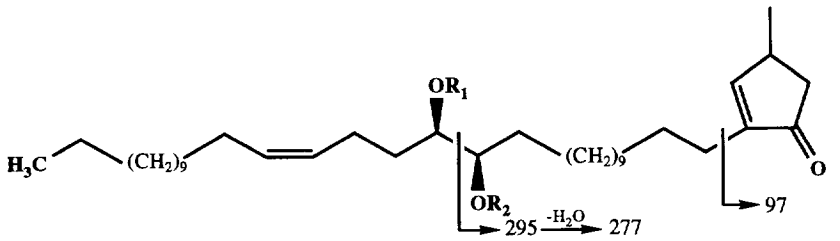
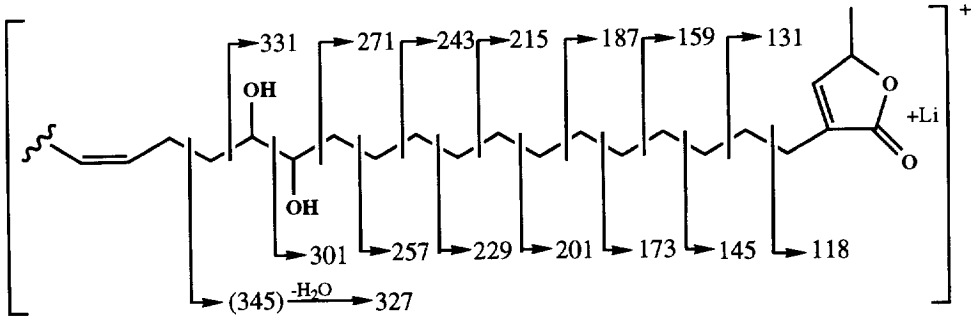


Fig. 3. Fragmentation (EI-mass spectrometry) of cohibin A (**1a**).



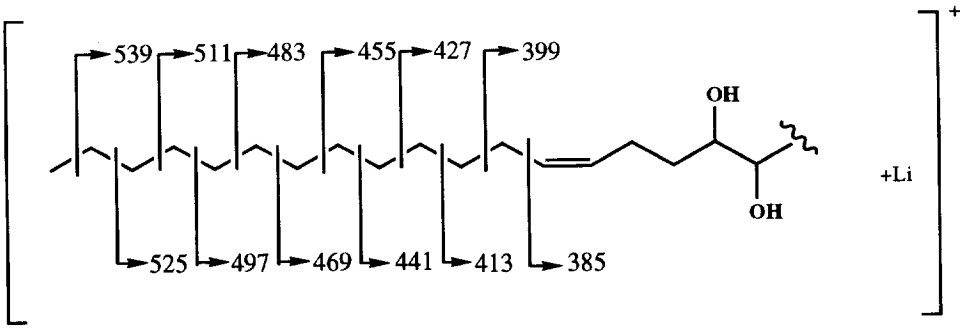


Fig. 4. Fragmentations tandem mass spectrometry of cohibin A (**1a**) A series; ( ) not observed.

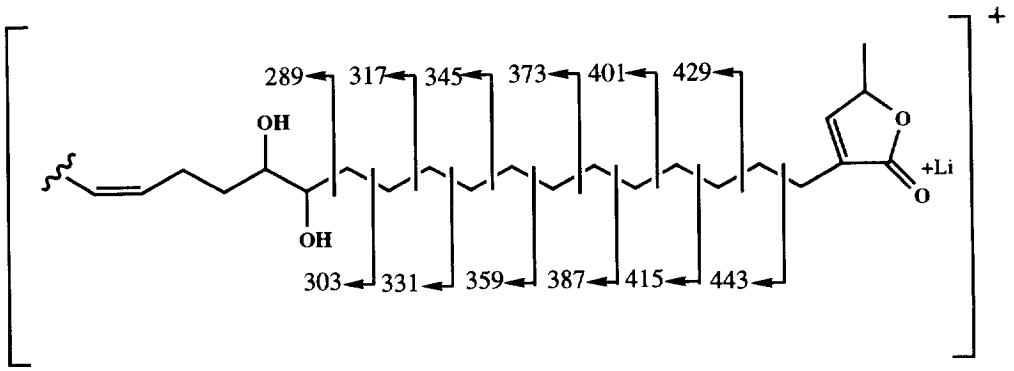


Fig. 5. Fragmentations tandem mass spectrometry of cohibin A (**1a**) corresponding B series.

these compounds displayed a single peak at δ 1.36 (6H) for the acetonyl methyl groups, while an overlapping multiplet at δ 3.59 determined a *trans*-stereochemistry for the ring. These observations allowed the assignment of a *threo*-configuration of the vicinal diol moiety [5].

Two acetogenins, cohibins A and B have thus been identified in the roots of *A. muricata*. In cohibin A, the diol system is located at C15–C16 and the double bond at C19–C20. In cohibin B, the diol system is at C13–C14 and the double bond at C17–C18 (Fig. 1). This type of acetogenin isomers could not be separ-

ated, even by HPLC [3, 13]; however, structural elucidation of this mixture could be achieved by tandem, mass spectrometry. The isolation of cohibins is also of particular interest with regard to the previously suggested biogenetic pathway leading to tetrahydrofuran acetogenins of the Annonaceae [14].

EXPERIMENTAL

General. Mps uncorr. Optical rotations were measured with a Schmidt-Haensch polartronic E. UV spectra were determined in MeOH on a Philips PU 8720 spectrophotometer. ^{1}H NMR spectra recorded at 200 and 400 MHz. ^{13}C NMR recorded at 50 MHz. EI-MS (48 eV) and CI-MS (CH_4). FAB-MS and a constant-B/E linked scanning expt were performed with a double-focusing instrument (Xe energy, 7 keV; matrix, *m*-nitrobenzyl alcohol + LiCl; collision gas, Ar; collision energy, 4 keV). MS/MS were obtained using five-sector tandem instrument. The first analyser (MS1) was triple sector ($E_1B_1E_2$) instrument and the second (MS_2) consisted of a double sector instrument (B_2E_3) of reverse Mattauch–Herzog geometry focusing the ion beam on a focal plane. $[M+Li]^+$ precursor ions were generated by Ce ion bombardment at 30 keV (matrix, *m*-NBA + LiCl). The precursor ions submitted to MS/MS experiments were selected by MS1 set at appropriate E and B values and then focused in a collision cell located in the fourth field-free region (between E_2 and B_2). He was introduced at a pres. leading to an attenuation of the precursor ion beam of *ca.* 70%. The collision cell was floated at 4 kV so as to attain a collision energy of 4 keV. Fragment ion detection was achieved by use of the MCAD detector operating with a mass ratio of 1.225:1.0 at an angle of 30° with regard to the ion beam [15–17]. For each MS/MS acquisition, the mass scale comprised between the precursor ion peak and the lowest mass end (*m/z* 50) was covered by successive overlapping exposures of 0.5 s. HPLC was performed with a pump (Waters 590), detector UV (Waters 84) and injector (Waters SSV).

Plant material. Roots of *A. muricata* L. were collected in Guinea (Conakry) in October 1993. A voucher specimen is deposited at the Faculty of Medicine and Pharmacy of Conakry.

Extraction and isolation. Dried and powdered roots (600 g) were extracted with MeOH to give a brown extract (60 g). The bioactive MeOH extract (brine-shrimp test positive [2]) was partitioned between H_2O and CH_2Cl_2 to yield 45 g of CH_2Cl_2 extract. This extract was subjected to silica gel CC Merck 230–400 mesh) and eluted with hexane containing increasing amounts of EtOAc. the frs collected were analysed by TLC (silica gel, Merck 60F254), on which basis they were grouped into 17 sets.

The solvent from fr. 7 was evapd off and the resulting residue (1.514 g) subjected to silica gel CC (Merck 60 H, 70–230 mesh) eluted with CH_2Cl_2–EtOAc, 20:3. Frs containing **1a** and **1b**, as judged from TLC, were combined and purified by prep. HPLC using a μBondapak C_{18} 10μm (250 × 20 mm) cartridge column, flow rate 9 ml min^{-1}, 20 mg per injection and MeOH–H_2O (23:2); 14 mg of **1a** and **1b** was obtained.

Cohibins A and B (**1a** *and* **1b**). Powder, mp 60–62°. $[\alpha]_D$ +12° (*c* 0.1, MeOH). IR ν_{max}^{KBr} cm^{-1} (3 300, 2 900, 2 840, 1 740, 1 650, 1 470, 1 120, 1 080, 1 030). UV λ_{max}^{MeOH} nm (log ε) 212.2 (2.82). HRCI-MS (CH_4) *m/z* 549.4707 $[M+H]^+$ calc. 549.4693 for $[C_{35}H_{64}O_4+H]^+$. CI-MS (*m/z*, rel. int.): $[MH]^+$ 549, $[MH-H_2O]^+$ 531, $[MH-2H_2O]^+$ 513, 295, 267. EIMS: 548 $[M]^+$, 295, 277, 267, 249, 111, 97 (Fig. 3). MS/MS: Figs 4 and 5. ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 50 MHz): Table 1.

Cohibin acetonide (**2**). Compounds **1a** and **1b** (6.4 mg) were dissolved in 1 ml of benzene, to which was added 2,2-dimethoxypropane (50 μl) and a trace of *p*-toluenesulfonic acid. The mixt. was stirred under reflux for 90 min and 0.1 mg of K_2CO_3 added and the mixt. stirred for 4 hr at room temp. Extraction with CH_2Cl_2 gave **2** (4.8 mg). Cl-MS (CH_4) *m/z* 589 $[M+H]^+$. ^{1}H NMR ($CDCl_3$ 200 MHz): δ 6.98 (1H, *d*, *J* = 1.7 Hz, H-33), 5.36–5.38 (2H, *m*, H-19,20), 5.00 (1H, *dq*, *J* = 1.7 and 6.8 Hz, H-34), 3.59 (2H, *m*, H-15,16), 2.26 (2H, *t*, *J* = 7.8 Hz, H-3), 1.42 (3H, *d*, *J* = 6.8 Hz, H-35), 1.36 (6H, *s*, acetonide), 0.87 (3H, *t*, *J* = 6.60 Hz, H-32).

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