Pergamon

S0031-9422(96)00050-7

Phytochemistry, Vol. 42, No. 4, pp. 1081–1083, 1996

0031-9422/96 $15.00 + 0.00

EPOMUSENINS A AND B, TWO ACETOGENINS FROM FRUITS OF *ROLLINIA MUCOSA**

YUAN-YNG CHEN, FANG-RONG CHANG, HSIN-FU YEN† and YANG-CHANG WU‡

Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung 807, Taiwan, R.O.C.; †Chia-Yi Agricultural Experiment Station, Taiwan Agricultural Research Institute, Chia-Yi 600, Taiwan, R.O.C.

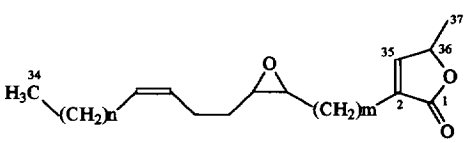
(*Received* 17 *November* 1995)

Key Word Index—*Rollinia mucosa*; Annonaceae; fruits; olefinic epoxy-acetogenins; epomusenins; rolliniastatin-1; rolliniastatin-2; squamocin.

Abstract—Chromatography of an ethyl acetate extract of the fruits of *Rollinia mucosa* led to the isolation of two new γ-lactone acetogenins, epomusenins A and B, which are the first examples of the rare C_{37}-type annonaceous acetogenins having an epoxide and a double bond along the aliphatic chain in place of the usual tetrahydrofuran moeity. The three known acetogenins, rolliniastatin-1, rolliniastatin-2 and squamocin were also isolated. The structures were established by means of one and two dimensional NMR experiments.

INTRODUCTION

Annonaceous acetogenins have attracted considerable attention as a rapidly growing class of new compounds; these natural polyketides, among other significant bioactivities, have excellent potential as new antitumour agents [1]. A number of cytotoxic acetogenins have been isolated so far from the leaves of *Rollinia mucosa* [2–4]. In previous papers, we have reported the presence of some cytotoxic acetogenins from *Annona reticulata* [5, 6] and *A. montana* [7]. As part of our ongoing studies on the acetogenins of Formosan Annonaceous plants, we have isolated a novel acetogenin, romusanin, which is the first adjacent *bis*-tetrahydrofuran ring with only one flanking OH, and possessing an unique β-pyrone ring moiety instead of traditional α,β-unsaturated γ-lactone ring acetogenin [8] and several acetogenins from the EtOAc extract of the fruits of *R. mucosa*. In this paper, we report the new rare C_{37}-skeleton acetogenin 'epomusenin' (**1**), which was isolated as an apparently pure compound. However, in the course of its structural determination, it became obvious that 'epomusenin' was not really pure, but was an unresolvable mixture of two isomeric acetogenins, named epomusenins A (**1a**) and B (**1b**), which contain one epoxy group and one double bond in place of the usual tetrahydrofuran ring. The three known compounds, rolliniastatin-1, rolliniastatin-2 and squamocin were also isolated. Up to now, only four of the C_{35}-type acetogenins, epomuricenins A and B and epoxymurins A and B, had been reported and provide important proof for the proposed biogenetic pathway for acetogenins containing one THF-ring [9, 10].



Epomusenin A (1a) : **m** = 14 ; **n** = 11

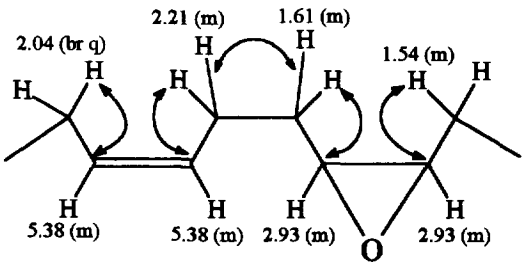
Epomusenin B (1b) : **m** = 12 ; **n** = 13

RESULTS AND DISCUSSION

Epomusenins were obtained as a wax. The strong absorption at 1760 cm^{-1} in the IR spectrum and a positive Kedde's reaction suggested the presence of an α, β-unsaturated γ-lactone, which was confirmed by comparison of the ^1H and ^{13}C NMR data with those of previously isolated acetogenins [9, 10] and a 2D NMR experiment. In addition to the signals corresponding to the methyl unsaturated lactone, the ^1H NMR spectrum showed characteristic chemical shifts for a long hydrocarbon chain (δ 1.26) with terminal methyl group (δ 0.87), but no carbinol methine protons and carbons are present indicating the absence of hydroxyl groups, and no signal related to the expected tetrahydrofuran moiety. The ^1H NMR spectrum exhibited two multiplets of two protons, at δ 2.93 and 5.38, which could be assigned to an epoxide and a double bond, respectively. In addition, the ^{13}C NMR spectrum showed the corresponding oxygen-bearing carbons of the epoxide at

*Part 6 in the series "Studies on the Acetogenins of Formosan Annonaceous Plants". For part 5, see ref. [8].

‡Author to whom correspondence should be addressed.



Scheme 1. Structural subunit **1a** and **1b**, assigned on the basis of ^{1}H-^{1}H COSY NMR.

δ 56.78 and 57.23, and the sp^3 carbons at δ 128.44 and 131.17. The presence of two adjacent methylene groups between the epoxide and the double bond was evidenced by ^{1}H-^{1}H correlations (Scheme 1).

The carbon skeleton and placement of the epoxide and the double bond of epomusenins were determined based on FAB-mass and EI-mass spectra analyses. In the FAB-mass spectrum, the isomeric protonated molecules at *m/z* 559 indicated a M_r of 558. Loss of a molecule of water from these gave an intense fragment at *m/z* 541, that was consistent with the presence of one epoxy group [9–11]. In the EI-mass spectrum (Scheme 2), a series of significant ion-peaks were observed between *m/z* 111 and 279, which contained the lactone and portions of the aliphatic chain. Besides these, the more intense and most significant ions resulted from fragmentation at the epoxy site, and they appeared as two series of fragment-ion peaks at *m/z* 323/295 (transannular cleavage), 335/307 (α-cleavage) and 336/308 (α-cleavage with hydrogen transfer). Other interesting fragments were observed at *m/z* 349/321 arising from β-cleavages to the epoxide [9–11]. Thus, all the fragments obtained by EI-mass spectrometry were consistent with the structures of the two isomers having the epoxide between the double bond and the lactone; Moreover, no fragmentation corresponding to the two other possible isomers could be detected in the spectrum.

Therefore, 'epomusenin', isolated from the fruits of *R. mucosa*, is a mixture of two isomeric acetogenins. In epomusenin A (**1a**) the epoxide is located at the C-17/C-18 and the double bond at the C-21/C-22 positions; in epomusenin B (**1b**) the epoxide is at the C-15/C-16 and the double bond at the C-19/C-20 positions along the hydrocarbon chain. The stereochemistry of the epoxide, however, could not be determined. For the double bond, selective irradiation at 400 MHz of the α-methylene group at δ 2.04 afforded a doublet for one of the olefinic protons, and the resulting coupling constant of 11 Hz was consistent with a Z-geometry of the double bond. This stereochemistry is in agreement with the ^{13}C NMR chemical shifts observed for the methylene carbons allylic to the double bond at 24.1 and 27.2; an E-geometry of this double bond would result in a significant downfield shift of these resonances [12].

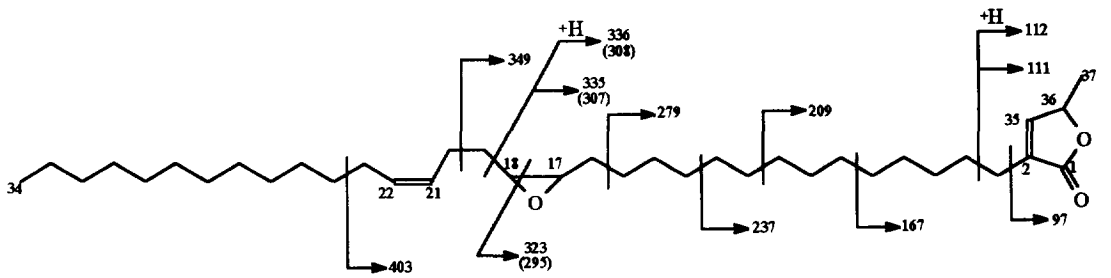
Rolliniastatin-1 [13], rolliniastatin-2 [6] and squamocin [13], were also isolated as oil-like substances and their identities were established by IR, mass spectrometric, ^{1}H and ^{13}C NMR studies [14] and, finally, by TLC comparison with authentic samples.

EXPERIMENTAL

General. UV spectra were obtained in EtOH, IR spectra as films. ^{1}H NMR (400 MHz), COSY (400 MHz) and ^{13}C NMR (100 MHz) spectra (all in $CDCl_3$) were recorded using TMS as int. standard. Low-resolution FAB-MS and low-resolution EI-MS were measured using a direct inlet system. TLC was performed on silica gel and spots were detected by spraying with Kedde's reagent and H_2SO_4.

Plant material. Fruits of *R. mucosa* Bail. were collected from Chia-Yi city, Taiwan, in June, 1994. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation. Fresh fruits (11 kg) were extracted repeatedly with EtOAc at room temp. The combined EtOAc extracts were evapd and partitioned to yield $CHCl_3$ and aq. extracts. The $CHCl_3$ layer after concn was partitioned between *n*-hexane and MeOH. After concn, the hexane soln afforded extract A (18.54 g) as an oil, positive to Kedde's reagent. The MeOH soln afforded extract B (30.60 g) as a wax, also positive to Kedde's reagent. The residual aq. layer afforded extract C (25.61 g), negative to Kedde's reagent. The hexane extract A was further separated by



Scheme 2. Fragmentations of epomusenin A under electron impact conditions. (Values in parentheses correspond to fragment-ion peaks of epomusenin B.)

CC on silica gel 60 (hexane–EtOAc, 8:1), the less polar frs contained a mixt. of fatty acids which were easily separated from epomusenins (**1a** + **b**) (6 mg) using exclusion chromatography on Sephadex LH-20 (CH_2Cl_2–MeOH, 4:1). From the MeOH extract B, were also isolated, by increasing solvent polarity, the three adjacent *bis*-tetrahydrofurane acetogenins rolliniastatin-1 (15 mg), rolliniastatin-2 (6 mg) and squamocin (12 mg).

Epomusenins (**1a** + **b**). Waxy solid. HRFABMS: $[M+1]^+$ at *m/z* 559.5090 (calc. for $C_{37}H_{67}O_{14}$, 559.5090). $[\alpha]_D^{25}$ +24° ($CHCl_3$; *c* 0.5). UV λ_{max}^{EtOH} nm (log ε): 210 (3.90). IR ν_{max}^{film} cm^{-1}: 2910, 2860, 1760, 1460, 1310, 1190, 1090, 1020, 715. ^1H NMR (400 MHz, $CDCl_3$) δ(numbering is given for epomusenin A): 6.98 (1H, *dd*, *J* = 1.6 and 1.6 Hz, H-35), 5.38 (2H, *m*, H-21, H-22), 5.00 (1H, *dqq*, *J* = 6.8, 1.6 and 1.6 Hz, H-35), 2.93 (2H, *m*, H-17, H-18), 2.27 (2H, *tdd*, *J* = 7.2, 1.6 and 1.6 Hz, H-3), 2.21 (2H, *br q*, H-20), 2.04 (2H, *br q*, H-23), 1.61 (4H, *m*, H-4, H-19), 1.51–1.56 (2H, *m*, H-16), 1.40 (3H, *d*, *J* = 6.8 Hz, Me-37), 1.21–1.39 (42H, *m*, H-5 to H-15 and H-24 to H-33), 0.87 (3H, *t*, *J* = 6.3 Hz, Me-34). ^{13}C NMR (100 MHz, $CDCl_3$) δ(epomusenin A): 174.15 (C-1), 149.01 (C-35), 134.52 (C-2), 131.17 (C-22), 128.44 (C.21), 77.00 (C-36), 57.23 and 56.78 (C-17 and C-18), 31.77 (C-32), 27.71–29.49 (C-4 to C-15 and C-24 to C-31), 27.23 (C-23), 27.07 (C-19), 26.45 (C-16), 25.01 (C-3), 24.14 (C-20), 22.51 (C-33), 19.03 (C-37), 13.91 (C-34); for epomusenin B, C-16 to C-23 became C-14 to C-21. FAB-MS *m/z*: 559 $[M+H]^+$, 541, 539, 445, 419, 367, 344, 237. EI-MS (probe) 70 eV *m/z*: 539, 524, 468, 417, 403, 368, 351, 349, 323, 321, 279, 265, 237, 209, 167, 141, 112, 111. 97.

Rolliniastatin-1. Waxy solid. $[\alpha]_D^{25}$ +26.4° ($CHCl_3$; *c* 0.1). IR ν_{max}^{film} cm^{-1}: 3470, 2930, 2860, 1750, 1600, 1450, 1310, 1070. EI-MS (probe) 70 eV *m/z*: 452, 433, 415, 397, 381, 363, 345, 311, 293, 241, 223, 171, 141, 123. Identified by direct comparison with authentic sample (IR, TLC, spectral data) and with lit. [13].

Rolliniastatin-2. Waxy solid. $[\alpha]_D^{25}$ +15.3° ($CHCl_3$; *c* 0.14). IR ν_{max}^{film} cm^{-1}: 3460, 2910, 2840, 1740, 1620, 1450, 1310, 1060. EI-MS (probe) 70 eV *m/z*: 452, 433, 415, 397, 381, 363, 345, 311, 293, 241, 223, 169, 141, 123. Identified by direct comparison with authentic sample (IR, TLC, spectral data) and with lit. [6].

Squamocin. Waxy solid, $[\alpha]_D^{25}$ +20° ($CHCl_3$; *c* 0.05). IR ν_{max}^{film} cm^{-1}: 3450, 2905, 2850, 1740, 1615, 1450, 1310, 1080. EI-MS (probe) 70 eV *m/z*: 604, 569, 519, 483, 436, 399, 365, 347, 329, 311, 295, 267, 239, 195, 169. Identified by direct comparison with authentic sample (IR, TLC, spectral data) and with lit. [13].

Acknowledgement—This investigation was supported by a grant from the National Science Council of the Republic of China (NSC 83-0208-M-037-009) awarded to Y. C. Wu.

REFERENCES

1. Fang, X.-P., Rieser, M. J., Gu, Z. M., Zhao, G. -X. and McLaughlin, J. L. (1993) *Phytochem. Anal.* **4**, 861.
2. Pettit, G. R., Cragg, G. M., Polonsky, J., Herald, D. L., Goswami, A., Smith, C. R. Moretti, C., Schmidt, J. M. and Weisleder, D. (1987) *Can. J. Chem.* **65**, 1433.
3. Pettit, G. R., Riesen, R., Leet, J. E., Polonsky, J., Smith, C. R., Schmidt, J. M., Dufresne, C., Schaufelberger, D. and Moretti, C. (1989) *Heterocycles* **28**, 213.
4. Shi, G., Zeng, L., Gu, Z. M., MacDougal, J. M. and McLaughlin, J. L. (1995) *Heterocycles* **41**, 1785.
5. Wu, Y. C., Chang, F. R., Duh, C. Y. and Wang, S. K. (1992) *Heterocycles* **34**, 667.
6. Chang, F. R., Wu, Y. C. and Duh, C. Y. (1993) *J. Nat. Prod.* **56**, 1688.
7. Wu, Y. C., Chang, F. R., Chen, K. S., Liang, S. C. and Lee, M. R. (1994) *Heterocycles* **38**, 1475.
8. Chen, Y. Y., Chang, F. R. and Wu, Y. C. (1995) *Tetrahedron Letters* (submitted).
9. Roblot, F., Laugel, T., Leboeuf, M., Cave, A. and Laprevote, O. (1993) *Phytochemistry* **34**, 281.
10. Hisham, A., Sreekala, U., Pieters, L., De Bruyne, T., Van den Heuvel, H. and Claeys, M. (1993) *Tetrahedron* **31**, 6913.
11. Brown, P., Kossanyi, J. and Djerassi, C. (1967) *Tetrahedron*, Suppl. 8, Part I, 241.
12. Rochfort, S., Murray, L. and Capon, R. J. (1992) *J. Nat. Prod.* **55**, 1332.
13. Wu, Y. C. and Chang, F. R. (1995) *Chemistry* (The Chinese Chem. Soc., Taiwan, China), **53**, 76.
14. Rupprecht, J. K., Hui, Y.-H. and McLaughlin, J. L. (1990) *J. Nat. Prod.* **53**, 237.