ISOLATION AND STRUCTURE OF ROLLINIASTATIN 2 : A NEW CELL GROWTH INHIBITORY ACETOGENIN FROM *ROLLINIA MUCOSA*[1]

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Abstract - A new cell growth inhibitory acetogenin, designated rolliniastatin 2, has been isolated from seeds of the South American *Rollinia mucosa* (Annonaceae) and its structure determined. A diastereomeric relationship for rolliniastatin 1, rolliniastatin 2, and asimicin was proposed.

The *Rollinia* genus (Annonaceae) is composed of some 65 species occurring principally from Central America to Argentina. The few species so far examined (chemically) from each of the *Annona*, *Asimina*, *Rollinia*, and *Uvaria* genera have been found to contain members of a new class of biologically active *bis*-tetrahydrofurans. Our crystal structure determination of rolliniastatin 1[2] provided the first definitive structure in this new series.[3-10] By x-ray crystallography, the relative stereochemistry of rolliniastatin 1 was established as 4*S**, 15*S**, 16*S**, 19*R**, 20*R**, 23*S**, 24*R**, and 36*R**.[2] Asimicin, recently isolated from *Asimina triloba*,[8] apparently differs from rolliniastatin 1 in its configuration at one or more of the chiral centers associated with the *bis*-tetrahydrofuran system.

Further detailed fractionation of a hexane extract of *Rollinia mucosa* seeds has led us to uncover companions of rolliniastatin 1, including asimicin and a new diastereomer of rolliniastatin 1, named rolliniastatin 2. Isolation of rolliniastatin 2 was effected by consecutive applications of solvent partitioning, flash chromatography on silica gel, high-speed countercurrent distribution (HSCCD)[11] with a multilayer coil planet centrifuge,[12] column chromatography (LiChrom Si60), and finally hplc (semipreparative with Partisil 10 silica gel followed by analytical with Partisil 5 silica gel) with the solvent system hexane - ethyl ether - methanol. With a hplc flow rate of 1.5 ml/min, emergence times (min) were: rolliniastatin 1, 6.17; asimicin, 6.57; rolliniastatin 2, 6.70.

High resolution sp-sims analysis of rolliniastatin 2 provided molecular formula $C_{37}H_{66}O_7$ (*m/z* 623.4885; calcd for $C_{37}H_{66}O_7$ + H, 623.4866). The uv, ir, and eims spectra of rolliniastatin 2 were similar to those reported for rolliniastatin 1,[2] asimicin,[8] and 14-hydroxy-25-desoxyrollinicin.[9] By thin-layer chromatographic comparison in dichloromethane - methanol (19:1), rolliniastatin 2 was hardly distinguishable

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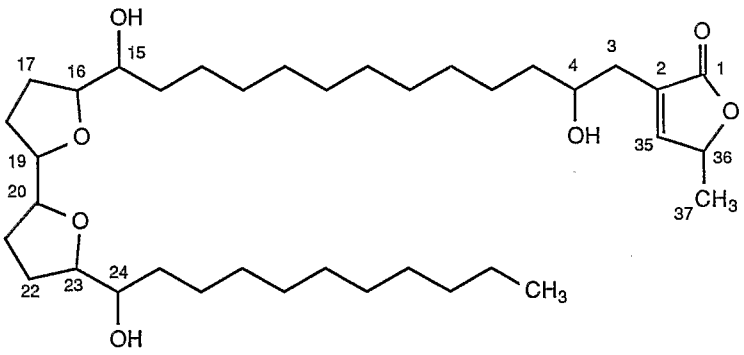
from asimicin. However, the 400 MHz ^{1}H- and ^{13}C- nmr spectra of rolliniastatin 2, especially shifts for carbons 19, 20, 21, and 22, were readily distinguishable from those of rolliniastatin 1 and asimicin. Distinct differences in the δ 1.5-2.1 region associated with methylene protons attached at C-17, C-18, C-21, and C-22 were noted. A diastereomeric relationship for the three acetogenins was deduced by comparing their ^{13}C-nmr spectra. Chemical shifts for carbons 1-6 and 35-37 were found to be virtually identical. Accordingly, the γ - lactone unit, carbons 3-6 in the side chain, and attachment of a hydroxyl group at C-4 were assumed to be the same in all three compounds. Confirmation was obtained by nmr studies that included $^{1}H,^{1}H$-COSY, $^{1}H,^{1}H$-relayed-COSY[13], and $^{1}H,^{13}C$-COSY experiments. Thus the same pattern of proton-proton and proton-carbon connections (Table 1) was established for rolliniastatin 2 as for rolliniastatin 1.[2] Although significant differences in shifts were observed for the *bis*-tetrahydrofuran systems, the three isomers apparently share the same regiochemical structure.



Rolliniastatin 1, 2, and asimicin.

Recently, Etse and Waterman[9] reported the isolation of 14-hydroxy-25-desoxyrollinicin from *Annona reticulata*. The structure was assigned primarily on the basis of eims fragmentation. When we compared the ^{13}C chemical shifts[14] of this compound with those of rolliniastatin 2, it became apparent that they may be identical. The average difference between pairs of observed shifts was only 0.05 ppm, and in only one case was the difference as much as 0.12 ppm. As noted previously with the structural determination of rolliniastatin 1,[2] eims fragmentation interpretations did not detect a hydroxyl group at C-4. While no specimen of 14-hydroxy-25-desoxyrollinicin was available for direct comparison, it now appears that the structure of this substance needs to be reassigned as a diastereomer of rolliniastatin 1.

Rolliniastatin 2 was evaluated against the P-388 lymphocytic leukemia cell line and showed an impressive ED_{50} of 2.3×10^{-4} μg/ml.[15] Further biological evaluation of rolliniastatins 1 and 2 as well as isolation and characterization of related cell growth inhibitory substances are in progress.

Table 1. Rolliniastatin 2 connectivities by high field (400 MHz) nmr in deuteriochloroform solution (δ in ppm downfield from TMS).

Chemical shifts connected			Carbon or proton connection		
A. Selected proton-carbon connections by H,C-COSY:					
δ 7.17	→	151.8	H-35	→	C-35
δ 5.05	→	77.9	H-36	→	C-36
δ 3.86	→	69.9	H-4	→	C-4
δ 2.52, 2.39	→	33.2	H-3a, H-3b	→	C-3
δ 1.41	→	19.1	H-37	→	C-37
B. Selected proton-proton connections by H,H- and H,H-relayed COSY:					
δ 7.17	→	5.05	H-35	→	H-36
δ 7.17	→	2.52, 2.39	H-35	→	H-3a, H-3b
δ 7.17	→	1.41	H-35	→	H-37
δ 5.05	→	1.41	H-36	→	H-37
δ 3.86	→	2.52, 2.39	H-4	→	H-3a, H-3b
δ 2.52	→	2.39	H-3a	→	H-3b

EXPERIMENTAL

General Experimental Procedures. All solvents employed for chromatography were redistilled. Thin layer chromatography (tlc) was performed on silica gel GHLF Uniplates (0.25 mm layer thickness) supplied by Analtech Inc., using dichloromethane - methanol (19:1) as mobile phase. Tlc results were interpreted under uv light and/or developed by anisaldehyde - acetic acid - sulfuric acid spray (heating at approximately 150 °C for 5 - 10 min). A Gilson Model FC-80 fraction collector was used with column chromatography. Melting points (uncorrected) were determined with a Kofler-type hot stage apparatus. Uv spectra were recorded using a Hewlett-Packard 8450A uv/vis spectrophotometer. The ^{1}H- and ^{13}C- nmr spectra (tetramethylsilane as reference standard in deuteriochloroform solution) were obtained with a Brüker AM-400 spectrophotometer. The mass spectral measurements were performed with an MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln, NB.

***Rollinia mucosa*.** Seeds of the *Rollinia mucosa* tree were collected in French Guiana. A voucher specimen ('herbier' no. CM927) is deposited with the Centre Orstom de Cayenne, Cayenne, French Guiana.

Extraction and Partitioning. Whole seeds (766 g) of *Rollinia mucosa* were ground to a coarse powder and extracted (vigorous stirring at ambient temperature for 18 hr) with hexane in portions using the ratio 150 g of seeds to 600-800 ml of hexane. The extraction was repeated twice. Solvent was removed *in vacuo* to yield a clear, light yellow oil (187 g). A portion of this oily hexane extract (11.6 g) was partitioned between hexane and methanol-water (9:1) to provide 9.85 g and 2.05 g respectively of hexane and aqueous methanol fractions.

Isolation of rolliniastatin 2. A 3.89 g aliquot of the methanol-water fraction was chromatographed (rapidly) on a column of silica gel. Elution with dichloromethane-methanol (97:3) led to seven fractions. The fourth and largest (1.59 g) was combined with a 1.52 g fraction similar in composition but prepared in a slightly different manner. The combined product was rechromatographed in the same manner with a solvent ratio of 99:1 to yield five fractions. The first two (0.964 g) were combined with fractions (1.095 g) of similar compositions processed by a more complex procedure. The pooled material (1.824 g) was further separated by HSCCD[11,12] in a series of experiments where 0.7 to 0.8 g of fraction at a time was separated using the solvent system heptane - ethyl acetate - ethanol - water (10:3:4:2). The coil (365 ml capacity) was operated at 800 rotations / min with a flow rate of 3 ml / min. Fractions found to contain primarily rolliniastatin 2 were combined (0.26 g) and further enriched by Lobar-type column chromatography (silica gel; size B). Elution with hexane - ethyl ether - methanol (6:2:1) at a flow rate of 6.0 ml / min afforded pure rolliniastatin 2 in fraction fifteen. The adjoining fractions from the Lobar column were subjected to semipreparative hplc on Partisil 10 (500 x 10.0 mm column) using hexane - ethyl ether - methanol (6:2:1) at 3 ml / min. Analytical hplc was performed with a Partisil 5 column (250 x 4.6 mm) and the solvent system hexane - ethyl ether - methanol (7:2:1) at a flow rate of 1.5 ml / min. By analytical hplc, relative emergence times (min) were found to be: rolliniastatin 1, 6.17; asimicin, 6.57; and rolliniastatin 2, 6.70. The total yield of pure rolliniastatin 2 was 23 mg. Recrystallization from chloroform gave a waxy solid melting at 73 - 76 °C; R_f 0.22 (dichloromethane - methanol 19:1); $[\alpha]_D^{27}$ + 5.3° (*c* 0.23, $CHCl_3$); uv λ_{max} (CH_3OH) (log ε) 210 (3.85); ir ν_{max} ($CHCl_3$) 3450, 2925, 2855, 1755, 1720 (sh), 1615, 1465, 1320 cm^{-1}; hreims, *m/z*: ($C_{37}H_{66}O_7$ molecular ion not observed), 604.4661 (0.2; M^+-H_2O; calcd for $C_{37}H_{64}O_6$ M-H_2O: 604.4703), 451.3109 (0.4), 433.2956 (6), 415.2860 (11), 381.2633 (3), 363.2560 (43), 345.2463 (19), 312.2296 (19), 311.2254 (100), 293.2252 (19); ^1H nmr (400 MHz): δ 0.87 (3H, t, *J* = 6.8 Hz, H-34), 1.25 (32 H, m, H-6 - 13, and 26 - 33), 1.42 (3H, d, *J* = 6.8 Hz, H-37), 1.46 (2H, m, H-5), 1.50 (2H, m, H-14), 1.7-2.0 (8H, m, H-17, 18, 21, and 22), 2.39 (1H, dddd, *J* = 15.1, 3.5, 1.6, and 1.5 Hz, H-3b), 2.52 (1H, dddd, 15.1, 3.5, 1.6, and 1.5 Hz, H-3a), 3.38 (1H, m, H-15), 5.05 (1H, ddd, *J* = 6.8, 1.5, and 1.5 Hz, H-36), 7.16 (1H, ddd, *J* = 1.5, 1.5, and 1.2 Hz, H-35); and ^{13}C nmr (100 MHz): 174.4 (C-1), 151.7 (C-35), 131.1 (C-2), 83.1 and 82.7 (C-16 and C-23), 82.4 and 82.2 (C-19 and C-20), 77.9 (C-36), 74.0 and 71.2 (C-15 and C-24), 69.9 (C-4), 37.3 (C-5), 33.4 (C-25), 33.2 (C-3 and C-14), 31.8 (C-32), 29.6 to 28.3 (C-7 to C-12, C-21, C-22, and C-27 to C-31), 28.8 and 28.3 (C-17 and C-18), 25.9 (C-6 and C-13), 25.5 (C-26), 22.6 (C-33), 19.0 (C-37), and 14.0 (C-34).

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