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ROLLINONE, A NEW CYTOTOXIC ACETOGENIN FROM *ROLLINIA PAPILIONELLA*

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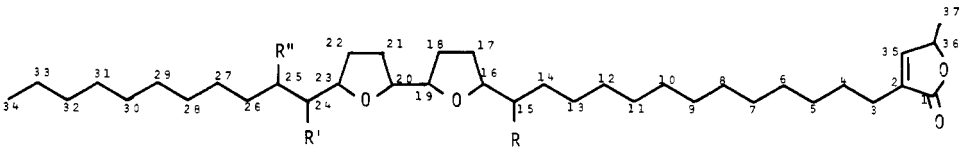
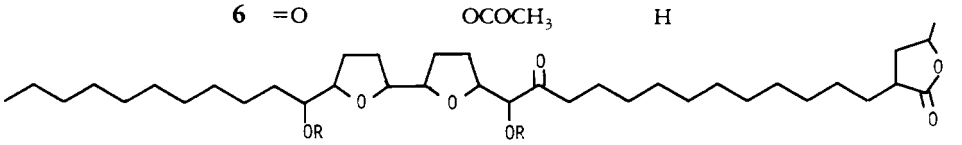
ABSTRACT.—Rollinone, a new member of a series of cytotoxic, linear acetogenins bearing a bistetrahydrofuran moiety and a γ-lactone, has been isolated from *Rollinia papilionella*. The structure elucidation of rollinone was achieved by interpretation of the pmr and cmr data and mass spectral fragmentation patterns.

Recent reports of the isolation of rollinicin (**1**) from *Rollinia papilionella* Diels (Annonaceae) and uvaricin (**2**) from *Uvaria accuminata* have introduced a new class of potential antineoplastic agents (1, 2). These linear acetogenins contain a bistetrahydrofuran moiety similar to that found in polyether antibiotics such as septamycin and antibiotic A28695B (3, 4), an α,β-unsaturated-γ-lactone like that found in marine natural products such as ancepsenolide (5) and two or more hydroxyl moieties. Continued work on the cytotoxic principles of *R. papilionella* has now resulted in the isolation and characterization of rollinone (**3**) a linear acetogenin closely related to **1** and **2**.

Fractionation of the ethanolic extract of *R. papilionella* guided by activity against the P-388 lymphocytic leukemia *in vitro* (6) resulted in the isolation and characterization of rollinicin (**1**) and isorollinicin (**4**) as previously reported (1). During this isolation procedure, several related principles were obtained in smaller amounts, and, in the course of the reisolation of additional amounts of **4**, one of these principles, rollinone (**3**), was also reisolated and characterized.



	R	R′	R″
1	OH	OH	OH
2	OH	$OCOCH_3$	H
4	OH	OH	OH
6	=O	$OCOCH_3$	H



3 R=H **5** R=$COCH_3$

Rollinone (**3**) was isolated as fine, colorless microcrystals (from EtOAc-hexanes) with a melting point of 54-56°. The molecular formula of rollinone (**3**) was established as $C_{37}H_{66}O_7$ by high resolution chemical ionization mass spectrometry [*m/z* 623.5078 (M^++H); calcd. for $C_{37}H_{66}O_7$+H, 623.4886]. Two successive losses of 18 mass units from the parent ion indicated the presence of two hydroxyl groups (*m/z* 605.4973 and *m/z* 587.4880). The presence of only two hydroxyl groups was confirmed by the presence of only two resonances due to carbons bearing hydroxyl moieties [72.3 ppm (d) and 74.1 ppm (d)] in the cmr spectrum of **3** and by the formation of a diacetate, **5**.[1]

[1]The diacetate of rollinone, **5**, gave an M^++H ion in the hrcims at *m/z* 707.5113 (cacld for $C_{41}H_{70}O_9$+H, 707.5098). Two successive losses of HOAc gave ions at *m/z* 647.4891 (calcd for $C_{39}H_{66}O_7$+H, 647.4886) and *m/z* 587.4686 (calcd for $C_{37}H_{62}O_5$+H, 587.4675). These ions served to further confirm the molecular formula of **3**.

The ir spectrum of rollinone (**3**) indicated the presence of a ketone (1726 cm^{-1}) in addition to a lactone (1780 cm^{-1}). Rollinone (**3**) formed a semicarbazone derivative and could be reduced with sodium borohydride to give a mixture of two triols, neither of which was identical to **1** or **4**. Rollinone also failed to give a positive Legal test (7), indicating that the α,β-unsaturated-γ-lactone moiety found in **1, 2,** and **4** was not present in **3**. The resonances for the vinyl proton, allylic proton, and allylic methyl group of the α,β-unsaturated-γ-lactone found in the spectra of **1, 2,** and **4** were absent in the pmr and cmr spectra of rollinone. Replacing these resonances in the pmr spectrum of **3** were a two-proton multiplet at δ2.6, a one-proton multiplet at δ4.40, and a three-proton doublet at δ2.18 (J=9 Hz), which indicated the presence of a saturated γ-lactone with a γ-methyl group. The cmr spectrum of **3** exhibited resonances at 23.2 (q), 36.8 (d), 78.6 (d), and 177.0 (s) ppm, which were also indicative of a saturated γ-methyl-γ-lactone ring, and the high resolution chemical ionization mass spectrum (hrcims) of **3** gave ions at *m/z* 113.0606 (6.5%) [$C_6H_9O_2$=113.0603] and *m/z* 99.0451 (5.1%) [$C_5H_7O_2$=99.0446], which support this assignment.

The remainder of the basic skeleton of **3** was determined from spectral data to be analogous to that of **1**. The bistetrahydrofuran moiety was indicated by an ion in the hrcims at *m/z* 141.0916 (9.1%) [$C_8H_{12}O_2$+H=141.0916] and by resonances in the cmr spectrum at 80.8 (d), 81.2 (d), 83.4 (d), and 83.8 ppm (d). A long alkyl chain was indicated by an unsymmetrical, three-proton triplet at δ0.88 coupled with a broad, intense signal at δ1.26 in the pmr of **3**. This chain was shown to be at least eight carbons in length by ions at *m/z* 85.1022 (4.9%, C_6H_{13}), 99.1170 (0.5%, C_7H_{15}), and

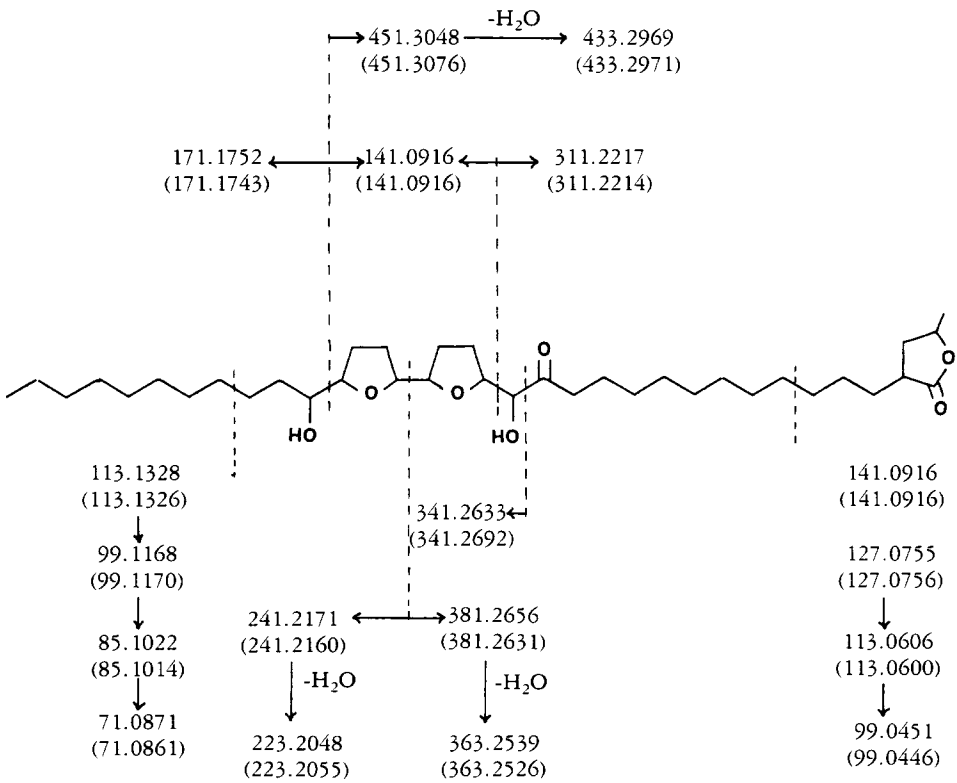


FIGURE 1. Mass spectral fragmentation pattern of rollinone (**3**) showing *m/z* ratios of major fragments (calculated *m/z* values in parentheses).

113.1326 (0.2%, C_8H_{17}) in the hrcims of **3**.[2] The accumulated data thus accounted for all but twelve -CH_2-groups. These -CH_2- groups were tentatively placed in the skeleton of **3** by analogy to **1**, **2**, and **4**.

The hrcims fragmentation pattern offered further support for the suggested carbon skeleton and limited the placement of the two hydroxyl groups and the ketone moiety. Major ions in the mass spectrum would be expected to arise via α-cleavage to carbons bearing oxygen. Examination of the hrcims fragmentation pattern of **3** (Figure 1) revealed ions at *m/z* 381.2656 (5.3%) [$C_{22}H_{37}O_5$=381.2641] and 241.2171 (9.7%) [$C_{15}H_{29}O_2$=241.2167], which arise from cleavage between C-19 and C-20; *m/z* 363.2539 (28%) [$C_{22}H_{35}O_4$=363.2535], which arises from loss of H_2O from the *m/z* 381 ion; and *m/z* 311.2217 (100%) [$C_{18}H_{31}O_4$=311.2222], which arises from the loss of the tetrahydrofuranyl ring (C_4H_6O) from the *m/z* 381 ion. Ions analogous to the *m/z* 381, 241, and 363 ions [*m/z* 423.2745 (100.0%), 283.2275 (29.9%), 363.2539 (58.9%)] were also detected in the hrcims of the diacetate of rollinone (**5**). The ion at *m/z* 381 confirmed that one of the tetrahydrofuran rings bore an eighteen-carbon substituent containing the lactone, one hydroxyl group, and the ketone moiety while the ion at *m/z* 241 indicated that the other tetrahydrofuran ring had an eleven-carbon substituent containing one hydroxyl group. Since the lactone ring must be one terminus of the molecule and the other terminus must be at least an eight-carbon chain, the positions of one hydroxyl group and the ketone were limited to between C-4 and C-15 and the second hydroxyl group to between C-24 and C-26.

The latter hydroxyl group was assigned to C-24 by analogy to **1**, **2**, and **4**. This was supported by ions at *m/z* 171.1752 (0.8%) [$C_{11}H_{23}O$=171.1749][3] and *m/z* 451.3048 (2.5%) [$C_{26}H_{43}O_6$=451.3076], which arise from fragmentation between C-23 and C-24. A major ion at *m/z* 493.3157 (20.2%) [$C_{28}H_{45}O_7$=493.3165] in the hrcims of **5** arising from the same fragmentation also supported this conclusion.

The positions of the remaining hydroxyl group and the ketone moiety were determined from inspection of the hrcims of **3** and **5**. The base ion resulting from cleavage between C-15 and C-16 in the hrcims of **3** was detected at *m/z* 311.2217 (100%, $C_{18}H_{31}O_4$), thus confirming that one of the two groups in question had to be at C-15. This ion then lost 58 mass units ($CO+CH_2O$) to give an ion at *m/z* 253 from which ten successive losses of 14 mass units were detected to give the ion at *m/z* 113 due to the lactone moiety. This fragmentation indicated that the two oxygen functions were at C-15 and C-14. A small ion at *m/z* 341.2633 (1.7%) [$C_{20}H_{37}O_4$=341.2692] was attributed to cleavage between C-14 and C-15 and suggested that the hydroxyl group was at C-15 and the ketone at C-14.

Additional support for this conclusion was obtained from a comparison of the pmr spectra of **3** and **5** with the published data for uvaricinone (**6**) (2), which has a ketone moiety at C-15. In the pmr spectrum of **6**, there is a 7 Hz triplet at δ4.40 assigned to the proton on the tetrahydrofuran ring at C-16 adjacent to the ketone moiety. There is no corresponding resonance in the spectra of **3** or **5**, indicating that the C-16 proton in **3** and **5** must be in a different environment from that of **6**. In fact, in the spectra of **3** and **5**, there is a broad multiple signal between δ3.8 and δ4.0 attributed to the four tetrahydrofuran protons indicating that they are in similar environments.

Further confirmation of this assignment was derived from the hrcims fragmentation pattern of **5** (Figure 2). Ions at *m/z* 425.2853 (8.1%) [$C_{24}H_{41}O_6$=425.2903] and *m/z*

[2]Normally, unbranched alkanes show a marked decline in ion intensity with increasing fragment weight such that the ions become difficult to detect, particularly when the fragments are larger than C_8H_{17} (8). In the case of **3**, small ions at *m/z* 127.1451 (0.1%) [C_9H_{19}=127.1482] and *m/z* 141.1634 (0.0%) [$C_{10}H_{21}$=141.1637] implied further that the chain length was at least ten carbons.

[3]An ion at *m/z* 153.1629 (0.2%) [$C_{11}H_{21}$=153.1637] indicated a loss of H_2O from the *m/z* 171 ion.

365.2684 (18.9%) [$C_{22}H_{37}O_4$=365.2692] suggested cleavage between C-14 and C-15 with C-15 bearing the acetate moiety followed by loss of HOAc from the initial ion. An ion at *m/z* 281.2140 (3.7%) [$C_{17}H_{29}O_3$=281.2117] was attributed to the fragment from C-1 through C-14 with C-14 bearing the ketone. Ions that would be expected from the opposite arrangement (ketone at C-15 and acetate at C-14) were not detected.

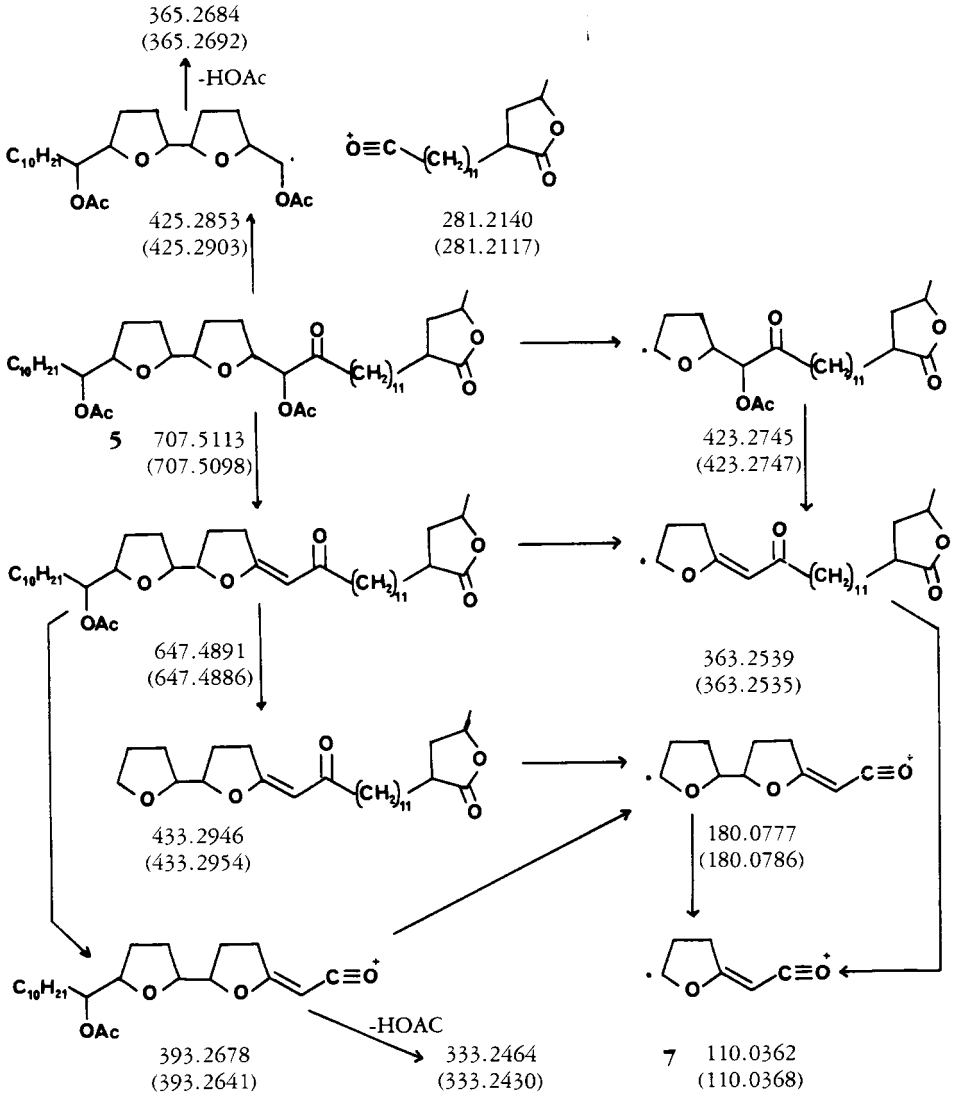
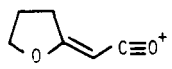


FIGURE 2. Mass spectral fragmentation pattern of **5** with *m/z* ratios of major fragments (calculated *m/z* values in parentheses).

In addition to these data, other ions in the hrcims of **5** (Figure 2) supported the conclusion that the acetate was at C-15 and the ketone at C-14. The base ion in the hrcims of **5** was found at *m/z* 423.2745 (100%) [$C_{24}H_{39}O_6$=423.2747] due to cleavage between C-19 and C-20. This ion then lost HOAc to give an ion at *m/z* 363.2539 (58.9%) [$C_{22}H_{35}O_4$=363.2539]. However, there was no ion observable that would result from the subsequent loss of the second tetrahydrofuranyl moiety. This indicated that further cleavage of the bond between C-15 and C-16 was not a favorable process. The most likely explanation for this would be that the acetate moiety was at C-15 and was elimi-

nated as HOAc in the mass spectral fragmentation process to give a double bond between C-15 and C-16. The unsaturated ketone would then undergo α-cleavage of the C-13,14 bond preferentially to give acylium ion **7** and an alkyl radical rather than α-cleavage of the C-14,15 bond, which would result in a vinyl radical (9). This conclusion was supported by an ion at *m/z* 110.0362 (2.9%) [$C_6H_6O_2$=110.0368] in the hrcims of **5** that was attributed to **7**. An ion at *m/z* 393.2678 (2.3%) [$C_{23}H_{37}O_5$=393.2641] was attributed to cleavage of the C-13,14 bond in the unsaturated ketone formed by elimination of the C-15 acetate moiety from **5** in the mass spectrotometer, and provided further evidence for the assignments. Therefore, based upon the mass spectral evidence, the second hydroxyl group of rollinone was assigned to C-15 and the ketone moiety to C-14, and the structure of rollinone was established as **3**.



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Rollinone (**3**) demonstrated cytotoxicity against the P-388 lymphocytic leukemia *in vitro* with an $ED_{50} < 10^{-5}$ μg/ml and activity *in vivo* against the P-388 lymphocytic leukemia in mice with a T/C=147% at 1.4 mg/kg (6). The activity of this class of acetogenins was unexpected based on their structural features and is being investigated further.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Ir spectra were measured on a Perkin-Elmer 283 instrument and uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. Pmr spectra were recorded on JEOL FX90Q spectrometer at 89.56 MHz or on a Nicolet 360 MHz spectrometer at the University of Virginia in $CDCl_3$ with TMS as an internal standard. Cmr spectra were recorded on a JEMOL FX90Q spectrometer at 22.5 MHz in $CDCl_3$ or C_6D_6 with TMS as an internal standard. High and low resolution cims were obtained at the University of Pennsylvania Mass Spectrometry Center. Microanalyses were carried out by Atlantic Microlab, Inc., Atlanta, Georgia. P-388 cytotoxicity assays were performed at Arthur D. Little, Inc., Cambridge, Massachusetts.

PLANT MATERIAL.—Roots of *R. papilionella* (B806512, PR-45518) were collected in Peru in October 1975, and were supplied by the Medicinal Plant Resources Laboratory, UDSA, Beltsville, Maryland, where voucher specimens are preserved.

ISOLATION OF ROLLINONE (**3**).—Dried, ground roots of *R. papilionella* (7.1 kg) were extracted in a Soxhlet extractor with 40 liters of 95% EtOH for 24 h. The resulting extract was concentrated *in vacuo* to give a brown solid (234 g) that was triturated with 1 liter of 2 N HCl. The insoluble material was partitioned between $CHCl_3$ (3×1000 ml) and H_2O (1000 ml), and the combined $CHCl_3$ layers were concentrated *in vacuo* to yield fraction A (71.2 g). A 41.2 g portion of fraction A was subjected to column chromatography over 800 g of silica gel 60 and eluted with $CHCl_3$ followed by increasing amounts of EtOH in $CHCl_3$. The fractions that eluted with 3% EtOH in $CHCl_3$ were combined and concentrated *in vacuo* to give fraction B (7.8 g). Fraction B was subjected to flash chromatography over silica gel 60 eluted with 5% *iso*-PrOH in $CHCl_3$ and the fractions shown to contain **3** by tlc were combined. These fractions were subjected to preparative tlc over silica gel 60 developed in diethylamine: *iso*-PrOH:toluene (2:5:93) to yield 55.7 mg of **3**.

Rollinone (**3**). Mp 54-55° (EtOAc-hexane): $[\alpha]^{25}$+25.0° (c 0.1371, $CHCl_3$); ir (CCl_4) 3486, 2930, 1780, 1726, 1466, 1410, 1356, 1050, 715 cm^{-1}; uv max (CH_2Cl_2) 231, 280 nm (ε 15199); pmr ($CDCl_3$) δ 0.88 (t, *J*=7 Hz, 3H, 34-CH_3), 1.26 (brs, ~34H), 1.47 (m, 3H), 1.7-2.0 (m, ~10H), 2.18 (d, *J*=9 Hz, 3H, 37-CH_3), 2.6 (m, 2H, 35-H_2), 3.05 (m, 1H), 3.08 (dt, *J*=18,3 Hz, 16-H), 3.42 (m, 1H), 3.8-3.95 (m, ~7H), 4.40 (m, 1H, 36-H); cmr (C_6D_6) ppm 14.4 q (C-34), 23.2 q (C-37), 24.4, 25.7, 26.7, 28.3, 28.6, 28.9, 29.4, 30.1, 32.4, 33.7, 35.0, 35.8 all t (C-3-12, 17, 18, 21, 22, 25-33, 35), 36.8 d (C-2), 43.8 (C-13), 72.3 d (C-15/24), 74.1 d (C-15/24), 78.6 d (C-36), 80.8 d, 81.2 d, 83.4 d, 83.8 d (C-16, 19, 20, 23), 177.0 s (C-1), 204.2 s (C-14); hrcims (isobutane reagent gas) *m/z* 623.5078 (calcd for $C_{37}H_{66}O_7$+H, 623.4886); cims *m/z* 623 (M^++H, 10.8%), 622 (0.4), 605 (9), 587 (9), 569

(7), 451 (2.5), 381 (5), 363 (28), 341 (1.7), 312 (21), 311 (100), 293 (13), 283 (0.5), 265 (5), 253 (1), 241 (10), 223 (3), 171 (1), 141 (9), 113 (7), 99 (5.1), 85 (5), 71 (7.6).

Anal calcd for $C_{37}H_{66}O_7$: C, 71.3; H, 10.6. Found: C, 69.9; H, 10.6.[4]

Rollinone diacetate (**5**). Rollinone (**3**) (13.2 mg) was treated with 1 ml Ac_2O-pyridine (1:1) at room temperature for 25 h. The mixture was dissolved in 5 ml CH_2Cl_2 and washed three times with 3 ml 1 N HCl followed twice by 3 ml H_2O. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to yield 10.2 mg of **5** as a clear oil: ir ($CHCl_3$) 3060, 2980, 2905, 1775, 1740, 1380, 1350, 820 cm^{-1}; pmr ($CDCl_3$) δ 0.88 (t, J=7 Hz, 3H, 34-CH_3), 1.25 (brs, ~34H), 1.5-2.0 (m), 2.05 (s, 3H, -$COCH_3$), 2.07 (s, 3H, -$COCH_3$), 2.19 (brs, 3H, 37-CH_3), 2.2-3.2 (m, 4H), 3.7-4.1 (m, 4H), 4.5 (m, 1H, 35-H), 4.9 (m, 2H, CH-OAc); hrcims (isobutane reagent gas) *m/z* 707.5113 (calcd for $C_{41}H_{70}O_9$+H, 707.5098); cims *m/z* 707 (M^++H, 4.0%), 647 (26.0), 587 (13.2), 493 (20.2), 425 (8.1), 423 (100.0), 393 (2.3), 365 (18.9), 363 (58.9), 353 (32.6), 333 (1.7), 311 (10.6), 293 (27.1), 283 (29.9), 281 (3.7), 253 (3.8), 223 (10.8), 180 (0.8), 141 (4.8), 127 (2.7), 113 (13.8), 110 (2.9), 99 (23.6), 85 (7.3), 71 (12.9).

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[4]The values calculated by taking into account one equivalent of EtOAc per two equivalents of 3 (C, 70.2; H, 10.6) match the found values somewhat better.