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BIOMIMETIC FORMATION OF A NIMBIN CLASS LIMONOID

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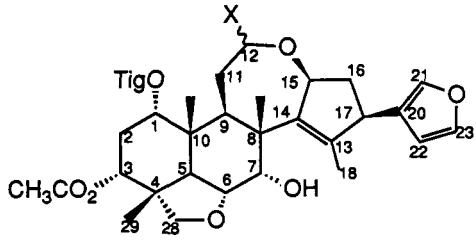
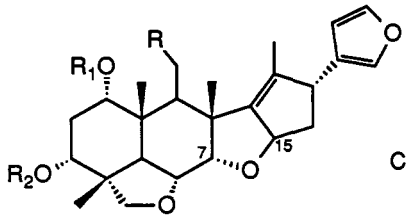
Salannin [**1**], previously isolated from the genera *Melia* and *Azadirachta*, belongs to a large and important group of frequently biologically active tetranortriterpenes of high structural complexity (1,2); however, little attention has been devoted to its biogenesis. During studies on the structure of the limonoid insect antifeedant volkensin [**2**] (3), a constituent of *Melia volkensii*, we discovered that treatment of a $CHCl_3$ solution of **2** with a 2.5% solution of trifluoroacetic acid in $CHCl_3$ induced a facile rearrangement to the hitherto unknown compound **3** in 85% yield within 30 min at room temperature. Mild oxidation of **3** with chromic acid in aqueous pyridine gave the corresponding acid **4**. Methylation of **4** with CH_2N_2 in Me_2CO yielded salannin [**1**], which was identified by comparison with an authentic sample (4).

A mechanism for the rearrangement that accounts for product stereochemistry at C-7 and C-15 would involve acid catalyzed C-ring opening of **2** to the 12-aldehyde **5** followed by rotation about the 8–14 bond and nucleophilic displacement by the 7α-OH on the protonated 15-OH. Molecular mechanics calculations (5) were employed to investigate the driving force for the **2** to **3** rearrangement. Program limitations on the number of input atoms required that an OH rather than an OAc be used at C-3 in the calculations; model calculations showed that this omission had no effect on the results. Direct comparison of the enthalpies of **2** and **3** is not feasible because these compounds contain different numbers of atoms as a molecule of H_2O is lost in the rearrangement. However, if one simply opens lactol **2** to the aldehyde/alcohol **5** and permits free rotation about the C-8 to C-14 bond, the resulting structure is comparable in stability to **2**. ΔH_f for the 12-α and 12-β epimers of **2** are −287.3 and −289.8 kcal/mole, respectively, while that of **5** is 288.7 kcal/mole. Furthermore, **5** adopts a conformation in which ring D lies at about a 30 degree angle to the plane of rings A–C, with C-15 just below the OH on C-7 and 3.1 Å away, an ideal situation for ring closure to **3**.

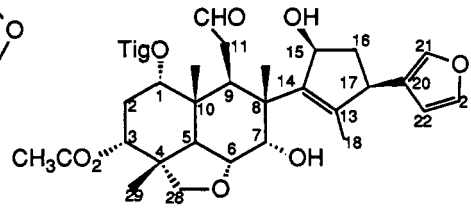
Taylor (2) has recently proposed a similar rearrangement as a key step in the biosynthesis of nimbin derivatives, but such a transformation has not until now been observed. According to the Taylor biosynthesis, **2** would arise by cleavage of the 12–13 bond of **6** and simultaneous opening of the epoxide to generate 12-CHO and 15-OH functions, with rotation about the 8–14 bond and recyclization leading to the lactol **2**. Rearrangement of **2** would then lead to **3**. Our observation of the latter rearrangement, together with isolation of **1** and **2** as major limonoids of the fruit of *M. volkensii* (3), lends support to Taylor's proposal. It is also possible, however, that oxidation at C-12 takes place before or concomitantly with rotation about the 8–14 bond and ring closure. We have, for example, observed such a rearrangement to the known nimbidic acid [**7**] (5) during acidic workup following base-induced cleavage of the C-ring lactone **8**. An alternate pathway to salannin from **9**, which involves cleavage of the 12–13 bond of **9** to form a 12-acylium ion followed by attack of the 7-OH on the 15 position, has been proposed by Mitra *et al.* (6), and a version of this pathway was recently included in a review by Siddiqui *et al.* (7). We feel that this proposal is not



2 X=OH
8 X==O



3 R=CHO, R_1=tiglate, R_2=Ac
4 R=COOH, R_1=tiglate, R_2=Ac
1 R=CO_2Me, R_1=tiglate, R_2=Ac
7 R=COOH, R_1=H, R_2=H



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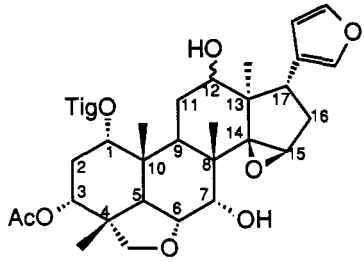

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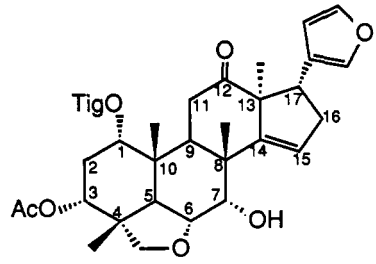

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mechanistically sound and, as presented, does not meet requirements for electronic bookkeeping.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher hot-stage apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 283 spectrometer. ^{1}H- and ^{13}C-nmr spectra were obtained on a Varian XL-200 system operating at 200 MHz for ^{1}H and 50.3 MHz for ^{13}C. ^{13}C multipicities were assigned using the APT technique. Low resolution mass spectra were recorded on an HP-5985 mass spectrometer operating at 70ev; high resolution mass spectra were obtained on a VG-70E system at the Auburn University Mass Spectrometry Facility by Dr. George Goodloe.

MOLECULAR MECHANICS.—Molecular mechanics calculations were performed with the Serena Software (POB 3076, Bloomington, IN 47402) version of MM2, QCPE No. 395.

REARRANGEMENT OF VOLKENSIN.—Volkensin [**2**] (200 mg) was dissolved in $CHCl_3$ (10 ml), and trifluoroacetic acid (5 drops, to make a ca. 2.5% solution) was added. The mixture was stirred at room temperature and the reaction monitored by Si gel tlc using Me_2CO-hexane (4:6). The reaction was essentially complete after 30 min, and the product mixture was immediately washed several times with 5% $NaHCO_3$ and finally with H_2O. The $CHCl_3$ was then dried with anhydrous Na_2SO_4 and decolorized with charcoal. The solvent was removed under reduced pressure and the resulting brown solid purified by cc using Si gel (70–230 mesh) eluted with Me_2CO-hexane (1:3) to give **3** as white needles (120 mg), mp 205–206° (from Me_2CO/hexane). Hrms yielded a parent ion at *m/e* 566.2903, corresponding to $C_{33}H_{42}O_8$. The ir

spectrum (KBr) displayed absorptions at 3135, 2950, 1740, 1710, 1655, 1500, 1260, 1150, 1050, 870, and 810 cm^{-1}. ^1H nmr (200 MHz, $CDCl_3$, TMS) δ 9.15 (1H, d, J = 4.8 Hz, H-12), 7.34 (1H, m, H-23), 7.14 (1H, m, H-21), 6.99 (1H, qq, J = 7, 1.4, H-3′ of tiglate), 6.12 (1H, m, H-22), 5.30 (1H, dd, J = 6,10, H-15), 4.99 (1H, t, J = 3, H-1), 4.86 (1H, t, J = 3, H-3), 4.20 (1H, d, J = 3.2, H-7), 4.00 (1H, dd, J = 3.2, 12.4, H-6), 3.73–3.58 (3H, m, H-28, H-17), 2.78 (1H, d, 12.4, H-5), 2.66 (1H, dd, J = 2.7, 10.5, H-9), 2.10–2.43 (6H, m, H-2, H-11, H-16), 1.96 (3H, s, OAc), 1.88 (3H, dq, J = 1,4,1, H-5′ of tiglate), 1.80 (3H, dq, J = 7,1, H-4′ of tiglate), 1.60 (3H, br s, H-18), 1.28 (3H, s, H-30), 1.23 (3H, s, H-29), 0.99 (3H, s, H-19); ^{13}C nmr ($CDCl_3$) δ 199.2 (C-12), 170.2 (carbonyl of acetate), 166.3 (carbonyl of tiglate), 145.1 (C-14), 142.7 (C-23), 137.6 (C-21 and C-3′), 135.9 (C-13), 128.2 (C-2′), 126.2 (C-20), 109.4 (C-22), 87.4 (C-15), 85.1 (C-7), 77.2 (C-28), 72.1 (C-1), 70.9 (C-3 and C-6), 49.0 (C-17), 48.1 (C-8), 42.2 (C-4), 41.3 (C-16), 40.0 (C-10), 39.5 (C-5 and C-11), 37.6 (C-9), 27.5 (C-2), 20.4 (acetal methyl), 19.0 (C-29), 16.3 (C-19), 14.9 (C-18), 13.9 (C-5′), 13.0 (C-30), 11.5 (C-4′). Primed numbers refer to the tiglate group.

OXIDATION OF **3**.—Compound **3** (100 mg) was dissolved in pyridine (5 ml) containing H_2O (2 drops), and an excess of chromic acid was added. The mixture was stirred at room temperature for 5 days. The pyridine was then evaporated using N_2 and the resulting brown solid purified by preparative tlc (25% $Me_2CO/CHCl_3$). The compound isolated was recrystallized from Me_2CO/hexane to give **4** as white needles, 40 mg, mp 258–260° (from Me_2CO/hexane). Low resolution ms yielded a parent ion at *m/e* 582. The ir spectrum (KBr) displayed absorptions at 3000 cm^{-1} br (3400–2500), 1715, 1730, 1655, 1260, 1045, 940, 870, 805. ^1H nmr (200 MHz, $CDCl_3$, TMS) δ 7.31 (1H, m, H-23), 7.19 (1H, m, H-21), 6.97 (1H, qq, J = 7,1.4, H-3′ of tiglate), 6.18 (1H, m, H-22), 5.41 (1H, dd, J = 6,10, H-15), 4.97 (1H, t, J = 3, H-1), 4.84 (1H, t, J = 3, H-3), 4.19 (1H, d, J = 3,2, H-7), 4.00 (1H, dd, J = 3.2,12.4, H-6), 3.70–3.56 (3H, m, H-28, H-17), 2.80 (1H, d, J = 12.4, H-5), 2.71 (1H, m, H-9), 2.30–2.10 (6H, m, H-2, H-11, H-16), 1.93 (3H, s, OAc), 1.88 (3H, dq, J = 1.4,1 H-5′ of tiglate), 1.78 (3H, dq, J = 7,1, H-4′ of tiglate), 1.68 (3H, br s, H-18), 1.31 (3H, s, H-30), 1.23 (3H, s, H-29), 1.01 (3H, s, H-19).

METHYLATION OF **4**.—Compound **4** (50 mg) was dissolved in Me_2CO (2.5 ml), and the solution was cooled to 0°. An excess of CH_2N_2 was added, and the reaction mixture was stirred at room temperature for 15 min. Si gel tlc analysis using Me_2CO-$CHCl_3$ (3:7) indicated absence of the starting material, and a new spot corresponding to salannin appeared. The solvent was then evaporated under vacuum, and the resulting white solid was shown by identity with published values (4,8) of the ^1H-nmr spectra, mass spectrum, ir spectrum, and melting point to be salannin [**1**].

REARRANGEMENT OF **8** TO NIMBIDIC ACID [**7**].—Compound **8** (100 mg) was dissolved in 5% MeOH/KOH (4 ml) and stirred for 3 h. The reaction mixture was then acidified with cold 6 N HCl until a white precipitate persisted. The acidified solution was then extracted several times with $CHCl_3$, and the combined $CHCl_3$ extracts were evaporated under reduced pressure to give a thick yellow oil. The crude product was then purified by preparative Si gel tlc using Me_2CO-hexane (1:1) containing 0.5 ml HOAc. The resulting white solid (30 mg) was shown by identity with published values (5) of the ^1H-nmr spectra, mass spectrum, ir spectrum, and melting point to be nimbidic acid [**7**].

ACKNOWLEDGMENTS

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