Trinervitene Diterpenes from Soldiers of Two *Nasutitermes* Species from French Guyana

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Methanolic extracts of soldiers of *Nasutitermes guayanae* and *N. surinamensis* have been shown to contain complex mixtures of diterpenes and monoterpenes. Eighteen diterpenes have been isolated and identified; twelve of them are previously known nasute termite diterpenes, while six are new trinervitene diterpenes. 2α,9β-Dihydroxy-3β,8β-oxido-1(15)-trinervitene (**1**) has been isolated from *N. guayanae*, while 3α,14α-diacetoxy-2β-hydroxy-1(15),8(19),9-trinervitatriene (**3**), 14α-acetoxy-2β,3α-dihydroxy-1(15),8(19),9-trinervitatriene (**4**), 2β,3α-diacetoxy-11β,14α-dihydroxy-1(15),8(19)-trinervitadiene (**5**), 9α,14α-diacetoxy-2β,3α-dihydroxy-1(15),8(19)-trinervitadiene (**6**), and 2β,9α,14α-triacetoxy-3α-hydroxy-1(15),8(19)-trinervitadiene (**7**) have been isolated from *N. surinamensis*. Their structures were determined on the basis of their spectroscopic properties.

Termites live in societies in which morphologically specialized individuals execute specific tasks: king and queen reproduce, workers forage and feed, and soldiers defend. Several defense mechanisms used by termite soldiers are based on the use of chemicals.[1] For example, a sticky solution that irritates and mechanically immobilizes small assailants may be ejected from a large frontal gland reservoir. This type of chemical defense is widely distributed in species of the subfamily Nasutitermitinae (Isoptera, Termitidae), in particular those of the genera *Nasutitermes* and *Trinervitermes*.[2] The soldiers of these genera indeed possess large cephalic pear-shaped frontal glands and a nozzle-like cephalic structure, called the nasus, from which they eject their defensive secretion. This secretion mainly consists of a mixture of cembrene-derived diterpenes dissolved in monoterpene hydrocarbons.[3] The diterpenes isolated so far from these secretions belong to the kempane,[4] longipane,[5] rippertane,[6] secotrinervitane,[7] or trinervitane[8] skeletons.

In our continuing search for defensive compounds from insects, we report the isolation and structure determination of six new diterpenes from termite soldiers of two not yet chemically investigated *Nasutitermes* species collected in French Guyana: *Nasutitermes guayanae* (Holngren) and *Nasutitermes surinamensis* (Holngren).

Results and Discussion

A GC-MS analysis of the methanolic extract of about 1000 soldiers of *N. guayanae* showed the presence of five monoterpene hydrocarbons, representing 41% of the total secretion. The compounds were identified as α-pinene (46.1% of the monoterpenic fraction), α-thujene (44.8%), β-pinene (6.4%), α-terpinene (1.8%), and α-phellandrene (0.9%) by comparison of their Kovats indices and their mass spectra with those reported in the literature.[9,10] Those monoterpenes are known to occur in several nasute termites.[9−12]

Five additional compounds were identified as diterpenes on the basis of their retention times and mass spectra,

accounting for the remaining 59% of the secretion. They were separated by successive column chromatographies on silica gel. Four of them were known termite diterpenes that were identified by comparison of their spectroscopic properties with those reported for 3α-hydroxy-15-rippertene, previously isolated from the secretion of several *Nasutitermes*[6,12−15] and *Hospitalitermes*[16−18] species and from *Longipeditermes longipes*,[5] 2α,3α-dihydroxy-1(15),8-(19)-trinervitadiene,[15,19−21] 2α,3β-dihydroxy-1(15),8-trinervitadiene,[20] and 2α,3β-dihydroxy-1(15),8(19)-trinervitadiene[19] previously found in several *Nasutitermes* species.

The ^{1}H NMR and ^{13}C NMR spectra of the fifth diterpene (**1**, $C_{20}H_{32}O_3$ as determined by HREIMS) of the secretion of *N. guayanae* indicated the presence of two secondary alcohol groups, one methyl group on a tetrasubstituted double bond, two tertiary methyl groups, one of which was located on a quaternary carbon atom bearing an oxygen atom, a secondary methyl group, and one further methine linked to an oxygen atom. All of these data suggested that **1** was a dihydroxylated 3,8-oxido-1(15)-trinervitene.[22] 2D NMR experiments (^{1}H/^{1}H COSY, HMQC, and HMBC) and comparison with the spectral properties reported for 2α-hydroxy-3β,8β-oxido-1(15)-trinervitene (**2**), isolated in 1983 from *Nasutitermes lujae*,[22] confirmed this hypothesis and allowed us to locate the hydroxyl groups at C-2 and C-9 and to assign all the NMR signals as presented in the Experimental Section. The relative configuration was fully established by NOESY experiments. Most noteworthy were the correlations observed between H-3 and H-18, between H-16 and H-7 and H-18, and between H-7 and H-9, H-17, and H-19, indicating that H-3, H-7, H-9, H-16, H_3C-17, H_3C-18, and H_3C-19 are on the same side of the molecule. Moreover, similar $J_{2,3}$ values were observed for **1** and the reference compound **2**.[22] It follows that **1** is 2α,9β-dihydroxy-3β,8β-oxido-1(15)-trinervitene. This is the second trinervitene possessing an ether bond linking C-3 to C-8 reported from a nasute termite.

GC-MS analysis of the methanolic extract of about 1000 soldiers of *N. surinamensis* showed the presence of a 1:1 mixture of two monoterpene hydrocarbons (33% of the terpenic fraction) identified as α-pinene and α-thujene on the basis of their Kovats indices and mass spectra.[9,10] A series of successive column chromatographies of the diter-

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pene fraction on silica gel yielded five new trinervitane diterpenes (**3**−**7**) together with 3α-hydroxy-1(15),8(19)-trinervitadien-2-one,[19,23] 3β-hydroxy-1(15),8-trinervitadien-2-one,[21] 3α-hydroxy-1(15),8(19)-trinervitadiene,[24,25] 2β-acetoxy-3α-hydroxy-1(15),8(19)-trinervitadiene,[5,16] 2β,3α-dihydroxy-1(15),8(19)-trinervitadiene,[19,25] 3α-hydroxy-1(15),8-(9)-trinervitadiene,[24] and 2β,3α-dihydroxy-1(15),8(9)-trinervitadiene,[5] which had already been isolated from various nasute termites. In addition, 3,4-epoxy-7,11,15-cembratriene[26,27] was also isolated. This is the first time that this compound is reported from a termite. Previously, it had been identified in various soft corals.[26−32] 3,4-Epoxy-7,11,15-cembratriene is structurally related to cembrene A, a trail-following pheromone found in some Nasutitermitinae.[33] All these compounds presented spectral properties identical to those reported in the literature.

1 R = OH
2 R = H

3 R = Ac
4 R = H

5

6 R = H
7 R = Ac
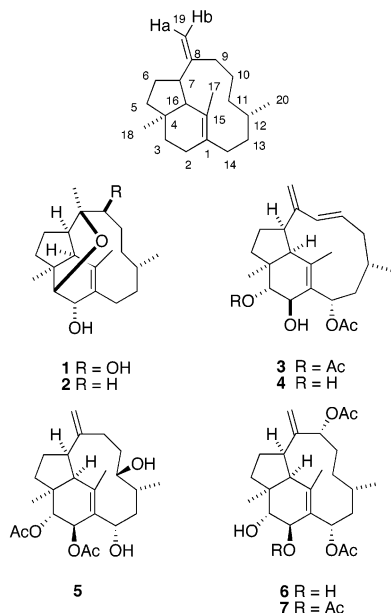
The diterpenes **3**−**7**, isolated from *N. surinamensis*, are novel compounds. Compound **3** was assigned the molecular formula $C_{24}H_{34}O_5$ by HREIMS. Its IR spectrum showed absorption bands for hydroxyl and acetoxy groups, and its UV spectrum (λ_{max} at 214 nm) is in agreement with the presence of a conjugated diene. The ^{1}H and ^{13}C NMR spectra showed the presence of two secondary acetoxy groups, one secondary alcohol, an exomethylene, a tetrasubstituted double bond, a *trans*-disubstituted double bond ($J_{9,10} = 16$ Hz), and one secondary and two tertiary methyl groups. All these data are compatible with a 1(15),8(19),9-trinervitatriene skeleton substituted by two acetoxy and one hydroxyl group. 2D NMR experiments (^{1}H/^{1}H COSY, HMQC, and HMBC) confirmed this hypothesis and allowed us to locate the hydroxyl group at C-2 and the acetoxy groups at C-3 and C-14, respectively. The relative configuration of **3** was determined by NOESY experiments. In particular, H-9 was correlated to H-3 and H-6β, suggesting that these hydrogens were all oriented toward the center of the molecule. Moreover, the exomethylenic hydrogen at δ 4.70 (H-19a) was correlated with H-7, while the one at δ 4.98 (H-19b) was correlated with H-10. These data supported an *s-cis* conformation for the diene moiety. No correlation was observed between H-3 and H-2 nor between H-3 and the H-18 hydrogens, suggesting a 3α-acetoxy and 2β-hydroxy *trans*-diaxial arrangement. Moreover, H-2 was correlated with H-14, which itself was correlated with the H-20 hydrogens, suggesting that this part of the 11-

membered ring adopts a conformation similar to that of the preferred conformation of 2β,3α,14α-triacetoxy-1(15),8-(19)-trinervitadiene.[15] From these data and the coupling constant values between H-14 and the vicinal methylene hydrogens H-13 ($J_{13\alpha,14} = 13$ Hz and $J_{13\beta,14} = 4.5$ Hz), we concluded that the relative configuration at C-14 was identical to that of 2β,3α,14α-triacetoxy-1(15),8(19)-trinervitadiene isolated from *Nasutitermes nigriceps* by Valterova et al.[15] Thus, compound **3** is 3α,14α-diacetoxy-2β-hydroxy-1(15),8(19),9-trinervitatriene.

The molecular formula of compound **4** was established as $C_{22}H_{32}O_4$ by HREIMS. Comparison of its spectroscopic properties with those of **3** indicated that it was the corresponding 3-deacetyl derivative. Their ^{1}H and ^{13}C NMR spectra were very similar, but the H-3 proton in **4** appeared at δ 3.68, whereas characteristic signals for only one acetoxy group were observed. HMQC, HMBC, and NOESY spectra confirmed this hypothesis as well as the relative configurations proposed for **3** and **4**. Moreover, when treated with a 1:1 mixture of pyridine/acetic anhydride, diterpene **4** yielded diacetate **3**. Thus the structure of **4** was unambiguously determined to be 14α-acetoxy-2β,3α-dihydroxy-1(15),8(19),9-trinervitatriene.

The molecular formula $C_{24}H_{36}O_6$ for compound **5** was deduced from a consideration of the exact mass, measured by HREIMS, of the fragment ion at *m/z* 360.2293 (M•+ − AcOH) and of the ^{13}C NMR spectrum. Its IR spectrum showed absorption bands for hydroxyl and acetoxy groups. The ^{1}H and ^{13}C NMR spectra confirmed the presence of two secondary acetoxy and two secondary hydroxyl groups and indicated the presence of an exomethylene, a tetrasubstituted double bond, and one secondary and two tertiary methyl groups. All of these data were compatible with a 1(15),8(19)-trinervitadiene skeleton bearing two acetoxy and two hydroxyl groups. The localization of the two acetoxy groups at the vicinal positions C-2β and C-3α clearly followed from the coupling constant ($J_{2,3} = 9$ Hz) and comparison of the chemical shifts (δ_{H-2} 5.77, δ_{C-2} 73.0, δ_{H-3} 5.33, δ_{C-3} 72.6) with those of compound **4** and of similar compounds in the literature.[23,25,34] 2D NMR experiments (^{1}H/^{1}H COSY, HMQC, and HMBC) allowed us to locate the two remaining hydroxyl groups at C-11 and C-14 as well as to assign all of the NMR signals as presented in the Experimental Section. Coupling constants and NOESY correlations similar to those reported for **3** and **4** were observed for H-14, H-13α, and H-13β in the NMR spectra of **5**. Thus, we located the second hydroxyl group at C-14 in the α-position. The determination of the relative configuration at C-11 was complicated by the flexibility of the 11-membered ring. However, the preferred conformation of this ring system had already been determined by X-ray diffraction analysis for related compounds, such as 9α-acetoxy-2β,3α-dihydroxy-1(15),8(19)-trinervitadiene.[8] We found that the NOESY correlations observed for **5** were in good agreement with this conformation. On the basis of these data, and the observation of a significant NOE correlation between the H-20 hydrogens and H-11, we suggest a β-position for the hydroxyl group at C-11. Consequently, compound **5** is 2β,3α-diacetoxy-11β,14α-dihydroxy-1(15),8(19)-trinervitadiene.

The molecular formula $C_{24}H_{36}O_6$ was assigned to compound **6** from a consideration of the exact mass, measured by HREIMS, of the fragment ion at *m/z* 360.2295 (M•+ − 60) and of the ^{13}C NMR spectrum. Its spectroscopic properties clearly showed that, like diterpene **5**, compound **6** was a 1(15),8(19)-trinervitadiene bearing two secondary hydroxyl groups and two secondary acetoxy groups. The

^{1}H and ^{13}C NMR spectra of **6** were very similar to those of **4**, at least with regard to the tetrahydroindane moiety of the molecule, indicating the presence of hydroxy groups at C-2β and C-3α and of an acetoxy group at C-14α. The second acetoxy group was located at C-9α considering the HMBC and NOESY correlations. In particular, H-9 was correlated with C-7, C-8, C-10, and C-19, and a clear NOE effect was observed between H-9 and H-3β. 2D NMR experiments confirmed these data and allowed us to assign all of the NMR signals. Thus, compound **6** is 9α,14α-diacetoxy-2β,3α-dihydroxy-1(15),8(19)-trinervitadiene.

The molecular formula $C_{26}H_{38}O_7$ was deduced from the HREIMS and ^{13}C NMR spectra of compound **7**. Comparison of its properties with those of **6** indicated that it was the corresponding 2-acetylated derivative of the latter. Indeed, their ^{1}H and ^{13}C NMR spectra (see Experimental Section) were very similar except that the H-2 signal of **7** appeared at δ 5.77 (δ 4.30 in **6**) and that characteristic signals for a third acetoxy group were observed. Thus, compound **7** is 2β,9α,14α-triacetoxy-3α-hydroxy-1(15),8(19)-trinervitadiene. Interestingly, H-14 is shielded from δ 5.52 in **6** to δ 4.96 in **7**. Such shielding has also been observed in another trinervitene derivative bearing acetoxy groups at C-2β and C-14α,[15] suggesting that it could be indicative for the simultaneous presence of acetoxy groups at these two positions.

In summary, like all the other *Nasutitermes* studied so far, the two species from French Guyana studied in this work secrete their own specific blend of cembrene-derived diterpenes dissolved in a monoterpenic solvent. It is generally recognized that the efficacy of a chemical defense system is greater for a multiple-component system than for a single-component system. The concerned species fulfill this requirement in a parsimonious way by making several functional variants of a single carbon skeleton, the 1(15),8-(19)-trinervitadiene skeleton, biosynthesized via transannular cyclization of a cembranoid precursor.[35]

Experimental Section

General Experimental Procedures. EIMS, HREIMS, and GC-EIMS analyses were performed with a Micromass Autospec 3F instrument (70 eV) coupled to a gas chromatograph equipped with a 25 m × 0.25 mm CP Sil 5 capillary column (Chrompack), at 40 °C (4 min), programmed to 200 °C at 8 °C min^{-1} (hold 5 min at 200 °C), then to 300 °C at 15 °C min^{-1} (hold 5 min); the carrier gas was helium. In all cases, peak intensities are expressed as percent relative to the base peak. The ^{1}H NMR spectra were recorded in CDCl$_3$ at 300 MHz with a Bruker Avance TM 300 or at 600 MHz using a Varian Unity 600 instrument and are reported in ppm from internal TMS on the δ scale. The ^{13}C NMR spectra were recorded in CDCl$_3$ at 75.4 MHz with a Bruker Avance TM 300 instrument. The IR spectra were recorded with a Bruker IFS 25 instrument as films on a NaCl disk and the UV/vis spectra with a Philips PU 8700 spectrophotometer in a 1 cm cell. Optical rotations were recorded at 589 nm (sodium D line) in a 1 dm cell at 20 °C on a Perkin-Elmer 141 polarimeter. GLC analyses were performed with a Varian 3400 apparatus equipped with a capillary column (30 m × 0.32 mm fused-silica column coated with OV1) at 100 °C (10 min), programmed to 200 °C at 5 °C min^{-1} (hold 30 min at 200 °C) (inj.: 110 °C; det.: 210 °C); the carrier gas was N$_2$. Thin-layer chromatography analyses (TLC) were performed with 0.25 mm Polygram silica gel SILG/UV254 precoated plates (Macherey-Nagel). Chromatographies were performed on silica gel columns (MN Kieselgel 60 0.04−0.063 mm) using the flash technique or on basic aluminum oxide (Macherey-Nagel) columns.

Extraction and Isolation. About 1000 specimens of soldiers of *N. guayanae* (Holngren) were collected around Petit Saut in French Guyana and dipped in MeOH. An aliquot of the methanolic solution was analyzed by GC-MS to identify the monoterpenes present in the extract and to evaluate the proportions of mono- and diterpenes. Kovats indices where determined by GC on an OV1 capillary column and calculated using C-8 to C-11 hydrocarbons. After filtration of the methanolic extract on cotton wool, the termites were crushed in fresh MeOH and the suspension filtered. The solid residue was then exhaustively extracted with MeOH (3 × 10 mL) and CH$_2$Cl$_2$ (3 × 10 mL). After evaporation of the combined organic phases, an oily residue (197.6 mg) was obtained. This crude extract was then submitted to silica gel column chromatography using as eluent a gradient from hexane (100%) to 7:3 hexane/ethyl acetate. This led to five fractions that were analyzed by ^{1}H NMR spectroscopy. F1 (12.3 mg) was composed of a mixture of fatty acid methyl esters, F2 (18.8 mg) contained 3α-hydroxy-15-rippertene, F3 (5.5 mg) contained a mixture of sterols, F4 (28.1 mg) contained 2α,3α-dihydroxy-1(15),8(19)-trinervitadiene, and F5 (21.4 mg) contained 2α,3β-dihydroxy-1(15),8-trinervitadiene, 2α,3β-dihydroxy-1(15),8(19)-trinervitadiene, and the new diterpene 2α,9β-dihydroxy-3β,8β-oxido-1(15)-trinervitene (**1**). The latter (4.1 mg) was further purified by two successive silica gel column chromatographies using the same eluents.

About 1000 specimens of soldiers of *N. surinamensis* (Holngren) were collected in French Guyana (Maroni) and dipped in MeOH. An aliquot of the methanolic solution was analyzed by GC-MS to identify the monoterpenes present in the extract and to evaluate the proportions of mono- and diterpenes. The Kovats indices where determined by GC on an OV1 capillary column and calculated using C-8 to C-11 hydrocarbons. After filtration of the methanolic extract on a cotton wool, the termites were crushed in fresh MeOH and the suspension filtered. The solid residue was further extracted with MeOH (3 × 10 mL) and CH$_2$Cl$_2$ (3 × 10 mL). After evaporation of the combined organic phases, an oily residue (500 mg) was obtained. This crude extract was then purified by successive chromatographies on silica gel and alumina columns, using mixtures of hexane/acetone, hexane/ether, hexane/ethyl acetate, and CH$_2$Cl$_2$/ethyl acetate as eluents. This led to the isolation of 13 diterpenes, eight of which were known derivatives, namely, 3α-hydroxy-1(15),8(19)-trinervitadien-2-one (**2**, 2.1 mg), 3β-hydroxy-1(15),8(9)-trinervitadien-2-one (8.8 mg), 3α-hydroxy-1(15),8(19)-trinervitadiene (11.5 mg), 2β-acetoxy-3α-dihydroxy-1(15),8(19)-trinervitadiene (6.3 mg), 2β,3α-dihydroxy-1(15),8(19)-trinervitadiene (119.4 mg), 3α-hydroxy-1(15),8-trinervitadiene (28.4 mg), 2β,3α-dihydroxy-1(15),8-trinervitadiene (31.9 mg), and 3,4-epoxy-7,11,15-cembratriene (3.2 mg). Their spectroscopic properties were identical to those described in the literature. The five other diterpenes, namely, 3α,14α-diacetoxy-2β-hydroxy-1(15),8(19),9-trinervitatriene (**3**, 63.8 mg), 14α-acetoxy-2β,3α-dihydroxy-1(15),8(19),9-trinervitatriene (**4**, 19 mg), 2β,3α-diacetoxy-11β,14α-dihydroxy-1(15),8-(19)-trinervitadiene (**5**, 2.1 mg), 9α,14α-diacetoxy-2β,3α-dihydroxy-1(15),8(19)-trinervitadiene (**6**, 12.6 mg), and 2β,9α,-14α-triacetoxy-3α-hydroxy-1(15),8(19)-trinervitadiene (**7**, 4.7 mg), are new compounds.

2α,9β-Dihydroxy-3β,8β-oxido-1(15)-trinervitene (1): IR ν_{max} 3416, 2950, 1661, 1652, 1645, 1634, 1456, 1375, 1037 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 4.22 (s, H-2), 3.63 (s, H-3), 1.49 (m, H-5α), 1.95 (m, H-5β), 1.52 (m, H-6α), 1.90 (m, H-6β), 2.40 (bt, J = 4 Hz, H-7), 4.50 (d, J = 10 Hz, H-9), 2.12 (m, H-10α), 1.32 (m, H-10β), 1.40 (m, H-11a), 1.41 (m, H-11b), 1.52 (m, H-12), 1.50 (m, H-13a), 1.78 (m, H-13b), 2.59 (dd, J = 14, 5 Hz, H-14α), 2.12 (m, H-14β), 1.89 (d, J = 4 Hz, H-16), 1.82 (s, H$_3$-17), 1.12 (s, H$_3$-18), 0.98 (s, H$_3$-19), 0.98 (d, J = 7 Hz, H$_3$-20); ^{13}C NMR (CDCl$_3$, 150 MHz) δ 131.0 (C-1), 74.7 (C-2), 83.5 (C-3), 36.5 (C-4), 36.2 (C-5), 26.2 (C-6), 41.9 (C-7), 78.7 (C-8), 71.8 (C-9), 33.9 (C-10), 34.9 (C-11), 37.1 (C-12), 36.7 (C-13), 29.6 (C-14), 133.9 (C-15), 52.9 (C-16), 19.7 (C-17), 23.1 (C-18), 20.3 (C-19), 27.6 (C-20); HREIMS m/z 320.2350 (M$^{•+}$, calcd for $C_{20}H_{32}O_3$, 320.2351), 302.2245 (M$^{•+}$ − H$_2$O, calcd for $C_{20}H_{30}O_2$, 302.2246).

3α,14α-Diacetoxy-2β-hydroxy-1(15),8(19),9-trinervitatriene (3): [α]$^{20}_D$ 36° (*c* 0.52, CHCl$_3$); UV (MeOH) λ_{max} (log

ϵ) 214 nm (14380); IR ν_{max} 3436, 2960, 2920, 2867, 1739, 1711, 1459, 1371, 1272, 1237, 1023 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 4.44 (m, H-2), 5.17 (d, $J = 9$ Hz, H-3), 1.21 (m, H-5α), 1.66 (m, H-5β), 1.76 (m, H-6α), 2.02 (m, H-6β), 3.10 (m, H-7), 6.00 (d, $J = 16$ Hz, H-9), 5.85 (ddd, $J = 16, 8, 5.4$ Hz, H-10), 1.66 (m, H-11α), 2.08 (m, H-11β), 1.98 (m, H-12), 2.23 (m, H-13α), 1.35 (ddd, $J = 13, 6, 4.5$ Hz, H-13β), 5.34 (dd, $J = 13, 4.5$ Hz, H-14), 2.23 (bd, $J = 10$ Hz, H-16), 1.73 (s, H$_3$-17), 1.00 (s, H$_3$-18), 4.70 (s, H-19a), 4.98 (s, H-19b), 0.98 (d, $J = 7$ Hz, H$_3$-20), 2.13 (s, Ac at C-3), 1.99 (s, Ac at C-14); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 130.3 (C-1), 70.6 (C-2), 77.0 (C-3), 45.8 (C-4), 36.3 (C-5), 31.1 (C-6), 49.3 (C-7), 150.2 (C-8), 132.2 (C-9), 129.8 (C-10), 38.2 (C-11), 29.7 (C-12), 38.9 (C-13), 70.9 (C-14), 134.8 (C-15), 58.9 (C-16), 20.8 (C-17), 21.7 (C-18), 110.2 (C-19), 23.7 (C-20), 172.4 and 21.3 (Ac at C-3), 170.8 and 21.2 (Ac at C-14); HREIMS m/z 402.2417 (M$^+$, calcd for C$_{24}$H$_{34}$O$_5$, 402.2406), 342.2165 (M^{+} − AcOH, calcd for C$_{22}$H$_{30}$O$_3$, 342.2195), 282.1989 (M^{+} − 2 AcOH, calcd for C$_{20}$H$_{26}$O, 282.1984).

14α-Acetoxy-2β,3α-dihydroxy-1(15),8(19),9-trinervitatriene (4): [α]$^{20}_D$ 51° (*c* 0.58, CHCl$_3$); UV (MeOH) λ_{max} (log ϵ) 215 nm (10100); IR ν_{max} 3449, 2953, 2866, 1737, 1713, 1455, 1372, 1249, 1051, 1021 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 4.26 (bd, $J = 9$ Hz, H-2), 3.68 (d, $J = 9$ Hz, H-3), 1.23 (m, H-5α), 2.08 (m, H-5β), 1.78 (m, H-6α), 1.87 (m, H-6β), 3.11 (m, H-7), 5.95 (d, $J = 15$ Hz, H-9), 5.84 (ddd, $J = 15, 8, 5$ Hz, H-10), 1.65 (m, H-11α), 2.07 (m, H-11β), 1.98 (m, H-12), 2.23 (m, H-13α), 1.32 (ddd, $J = 13, 6, 4$ Hz, H-13β), 5.34 (dd, $J = 13, 4$ Hz, H-14), 2.22 (bd, $J = 10$ Hz, H-16), 1.71 (d, $J = 1$ Hz, H$_3$-17), 0.97 (s, H$_3$-18), 4.68 (d, $J = 2$ Hz, H-19a), 4.94 (d, $J = 2$ Hz, H-19b), 1.00 (d, $J = 7$ Hz, H$_3$-20), 2.00 (s, Ac at C-14); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 129.9 (C-1), 72.6 (C-2), 73.6 (C-3), 46.0 (C-4), 36.2 (C-5), 31.5 (C-6), 50.0 (C-7), 150.5 (C-8), 132.2 (C-9), 129.3 (C-10), 37.7 (C-11), 29.8 (C-12), 38.5 (C-13), 70.9 (C-14), 135.3 (C-15), 58.9 (C-16), 20.8 (C-17), 20.8 (C-18), 109.8 (C-19), 23.3 (C-20), 171.2 and 21.3 (Ac at C-14); HREIMS m/z 360.2268 (M$^+$, calcd for C$_{22}$H$_{32}$O$_4$, 360.2301), 342.2177 (M^{+} − H$_2$O, calcd for C$_{22}$H$_{30}$O$_3$, 342.2195), 300.2068 (M^{+} − AcOH, calcd for C$_{20}$H$_{28}$O$_2$, 300.2089).

2β,3α-Diacetoxy-11β,14α-dihydroxy-1(15),8(19)-trinervitadiene (5): [α]$^{20}_D$ 21° (*c* 0.21, CHCl$_3$); IR ν_{max} 3422, 2951, 2873, 1732, 1455, 1372, 1243, 1023 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 5.77 (d, $J = 9$ Hz, H-2), 5.33 (d, $J = 9$ Hz, H-3), 1.21 (m, H-5α), 1.58 (m, H-5β), 1.71 (m, H-6α), 1.88 (m, H-6β), 3.19 (ddd, $J = 12, 12, 8$ Hz, H-7), 1.88 (m, H-9a), 2.11 (m, H-9b), 1.58 (m, H-10a), 2.12 (m, H-10b), 4.82 (bd, $J = 11$ Hz, H-11), 1.90 (m, H-12), 2.39 (bdd, $J = 13, 13$ Hz, H-13α), 1.26 (m, H-13β), 4.14 (dd, $J = 13, 4.5$ Hz, H-14), 2.48 (d, $J = 12$ Hz, H-16), 2.07 (bs, H$_3$-17), 1.11 (s, H$_3$-18), 4.97 (s, H-19a), 4.79 (s, H-19b), 0.96 (d, $J = 7$ Hz, H$_3$-20), 2.05 (s, Ac at C-2), 2.04 (s, Ac at C-3); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 130.2 (C-1), 73.0 (C-2), 72.6 (C-3), 46.4 (C-4), 36.5 (C-5), 30.4 (C-6), 52.8 (C-7), 151.6 (C-8), 25.0 (C-9), 31.9 (C-10), 78.0 (C-11), 28.9 (C-12), 37.1 (C-13), 68.1 (C-14), 138.2 (C-15), 58.0 (C-16), 22.4 (C-17), 21.2 (C-18), 113.6 (C-19), 19.8 (C-20), 171.0 and 21.3 (Ac at C-2), 171.0 and 21.0 (Ac at C-3); HREIMS m/z 420 (M$^{+\bullet}$) not detected, 360.2293 (M$^{+\bullet}$ − AcOH, calcd for C$_{22}$H$_{32}$O$_4$, 360.2301), 300.2073 (M$^{+\bullet}$ − 2 AcOH, calcd for C$_{20}$H$_{28}$O$_2$, 300.2089), 282.1979 (M^{+} − 2 AcOH − H$_2$O, calcd for C$_{20}$H$_{26}$O, 282.1984).

9α,14α-Diacetoxy-2β,3α-dihydroxy-1(15),8(19)-trinervitadiene (6): [α]$^{20}_D$ 44° (*c* 0.61, CHCl$_3$); IR ν_{max} 3471, 2954, 2924, 2866, 1731, 1368, 1246, 1019 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 4.30 (m, H-2), 4.03 (d, $J = 9$ Hz, H-3), 1.13 (m, H-5α), 2.05 (m, H-5β), 1.66 (m, H-6a), 1.68 (m, H-6b), 3.18 (q, $J = 12$ Hz, H-7), 5.54 (d, $J = 10$ Hz, H-9), 1.72 (m, H-10a), 1.83 (m, H-10b), 1.06 (m, H-11a), 1.20 (m, H-11b), 1.94 (m, H-12), 2.08 (m, H-13α), 1.36 (m, H-13β), 5.52 (dd, $J = 13, 5$ Hz, H-14), 2.30 (d, $J = 13$ Hz, H-16), 1.82 (d, $J = 2$ Hz, H$_3$-17), 0.96 (s, H$_3$-18), 5.17 (s, H-19a), 5.22 (s, H-19b), 0.96 (d, $J = 6$ Hz, H$_3$-20), 2.02 (s, Ac at C-9), 2.00 (s, Ac at C-14); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 133.6 (C-1), 73.3 (C-2), 72.2 (C-3), 46.6 (C-4), 36.3 (C-5), 31.1 (C-6), 52.1 (C-7), 151.9 (C-8), 69.0 (C-9), 32.6 (C-10), 33.0 (C-11), 25.2 (C-12), 37.6 (C-13), 70.7 (C-14), 133.7 (C-15), 57.4 (C-16), 22.0 (C-17), 20.1 (C-18), 117.8 (C-19), 23.4 (C-20), 171.1 and 21.4 (Ac at C-9), 170.6 and 21.1 (Ac at C-14); HREIMS m/z 420 (M$^{+\bullet}$) not detected, 360.2295 (M$^{+\bullet}$ − AcOH,

calcd for C$_{22}$H$_{32}$O$_4$, 360.2301), 300.2074 (M$^{+\bullet}$ − 2 AcOH, calcd for C$_{20}$H$_{28}$O$_2$, 300.2089), 282.1976 (M$^{+\bullet}$ − 2 AcOH − H$_2$O, calcd for C$_{20}$H$_{26}$O, 282.1984).

2β,9α,14α-Triacetoxy-3α-hydroxy-1(15),8(19)-trinervitadiene (7): [α]$^{20}_D$ 59° (*c* 0.47, CHCl$_3$); IR ν_{max} 3484, 2953, 2924, 2866, 1732, 1367, 1242, 1019 cm^{-1}; ^{1}H NMR (CDCl$_3$, 600 MHz) δ 5.77 (bd, $J = 9$ Hz, H-2), 4.14 (d, $J = 9$ Hz, H-3), 1.14 (m, H-5α), 2.08 (m, H-5β), 1.66 (m, H-6α), 1.69 (m, H-6β), 3.21 (q, $J = 12$ Hz, H-7), 5.48 (d, $J = 10$ Hz, H-9), 1.71 (m, H-10a), 1.86 (m, H-10b), 1.06 (m, H-11a), 1.28 (m, H-11b), 2.04 (m, H-12), 2.08 (m, H-13α), 1.36 (m, H-13β), 4.96 (dd, $J = 12, 5$ Hz, H-14), 2.37 (d, $J = 12$ Hz, H-16), 1.87 (d, $J = 2$ Hz, H$_3$-17), 1.03 (s, H$_3$-18), 5.19 (s, H-19a), 5.24 (s, H-19b), 0.99 (d, $J = 7$ Hz, H$_3$-20), 2.22 (s, Ac at C-2), 2.02 (s, Ac at C-9), 1.99 (s, Ac at C-14); ^{13}C NMR (CDCl$_3$, 75 MHz) δ 130.0 (C-1), 77.4 (C-2), 71.2 (C-3), 47.4 (C-4), 36.1 (C-5), 30.7 (C-6), 52.1 (C-7), 151.7 (C-8), 68.8 (C-9), 32.6 (C-10), 33.0 (C-11), 25.0 (C-12), 37.6 (C-13), 70.7 (C-14), 136.6 (C-15), 57.3 (C-16), 22.4 (C-17), 20.3 (C-18), 118.0 (C-19), 23.7 (C-20), 172.7 and 21.6 (Ac at C-2), 170.7 and 21.4 (Ac at C-9), 170.3 and 20.9 (Ac at C-14); HREIMS m/z 462 (M$^{+\bullet}$) not detected, 402.2392 (M$^{+\bullet}$ − AcOH, calcd for C$_{24}$H$_{34}$O$_5$, 402.2406), 342.2185 (M$^{+\bullet}$ − 2 AcOH, calcd for C$_{22}$H$_{30}$O$_3$, 342.2195), 282.1976 (M$^{+\bullet}$ − 3 AcOH, calcd for C$_{20}$H$_{26}$O, 282.1984).

Acetylation of Compound 4. Diterpene **4** (5.7 mg) was dissolved in a 1:1 mixture of pyridine/acetic anhydride (2 mL). After stirring the solution for 24 h at room temperature, ethanol (4 mL) was added and the solvent removed under reduced pressure. The solid residue thus obtained was purified by flash chromatography on a silica gel column (eluent: hexane/AcOEt, 95:5) to afford a white solid (5.4 mg) identified as diterpene **3** by direct comparison with an authentic sample.

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