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DITERPENIC STRESS METABOLITES FROM CASSAVA ROOTS

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Key Word Index—*Manihot esculenta*; Euphorbiaceae; Cassava root; stress metabolite; oxygenated diterpene; *ent*-beyerane type; *ent*-atisane type; *ent*-pimarane type; *ent*-kaurane type.

Abstract—Twenty-two diterpenic stress metabolites, most of which are novel, have been isolated and identified from cassava (*Manihot esculenta*) root tissues damaged by cutting or fungal-infection. The metabolites can be classified into four families; *ent*-beyerane (10 components), *ent*-pimarane (9 components), *ent*-atisane (2 components) and *ent*-kaurane (1 component). Diterpenes are not common as stress metabolites in plants.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) roots are one of the staple foods in the tropics and are also utilized as raw materials for starch production and alcohol fermentation. While this crop has many advantages, it has two major defects, namely, the occurrence of poisonous cyanogenic glucosides in the tissue and the high perishability of the roots which begin to deteriorate physiologically or microbially soon after harvest. This deterioration lowers the food quality and causes a serious problem. When cassava root is damaged by cutting (physiologically) or by fungal-infection (microbially), the tissues accumulate several abnormal secondary metabolites such as coumarinic (scopoletin, scopolin and esculin) and phenolic (catechin) compounds in the injured or infected regions [1].

Recently we reported that various types of stress metabolites were produced in the cassava root tissue damaged by cutting and fungal-infection; the induced stress compounds are predominantly steroids and diterpenoids [2]. In this paper we report the isolation and structural determination of 22 diterpenic stress metabolites (DSM), most of which are novel, from damaged cassava roots.

RESULTS AND DISCUSSION

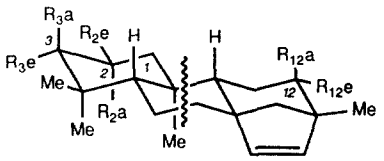
By means of exhaustive chromatographic separation (CC and HPLC) of the DSM, three known diterpene hydrocarbons, 16 diterpenes with two to three oxygen functions which were detected by GC/MS analysis [2] and three additional diterpenes with four oxygen functions were isolated from the methanol extract of the damaged part of cassave roots (cv 'Golden Yellow' grown in the Philippines).

The structures of the DSM were established by IR, mass spectrometry (EI and CI), NMR (^{1}H and ^{13}C) and UV spectroscopic studies. Mass spectral data indicated the molecular formula of each component, whereas IR and ^{13}C NMR spectra revealed the nature of the oxygen functions. In particular, two-dimensional proton–proton shift correlation spectroscopy (COSY) was the most powerful method for structure elucidation. Inspection of the 2D contour maps clarified the proton–proton correlation of continuous spin systems within the A/B ring or the C/D ring moiety (structural element). The presence of cross peaks due to *W*-type long-range couplings, 1, 3-diaxial couplings, allylic couplings, or homoallylic couplings, defined the streochemistry of the elements. All oxygenated DSM were made up of a combination of the two structural elements due to the A/B ring (7 types: elements **H–K**) and the C/D ring (10 types: elements **A–G**) moieties. These DSM can be classified into four typical diterpene families, viz. *ent*-beyerane (10 components), *ent*-pimarane (9 components), *ent*-atisane (2 components) and *ent*-kaurane (1 component). They were tentatively designated as yucalexins **B, P, A,** and **K** and numbered in order of increasing polarity after silica gel column chromatography.

ent-*Beyerane family*

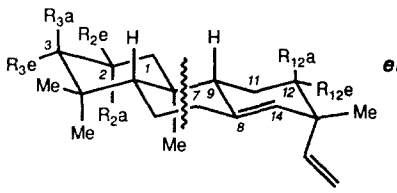
The largest group of DSM is the *ent*-beyerane family consisting of yucalexins **B-5,-6,-7,-9,-14,-18,-20,** and **-22** together with the known (+) -stachene (**B-1**). A 3-norbeyerene derivative **B′-11** is also included here. ^{1}H NMR spectra revealed the presence of two sets of characteristic AB type quartets due to the geminal methylene protons at C-14 and due to two vicinal olefin protons at C-15 and C-16 in the C/D ring moieties (elements **A, B,** and **B′**).

Compound **B-9** is the most abundant DSM constituent and one of the bitter principles of the damaged tissue. The molecular formula of **B-9** was determined to be $C_{20}H_{28}O_3$ by high resolution mass spectrometry. The spectral data showed **B-9** to be an *ent*-beyerene derivative with one ketone and one α-ketol group built up from elements **A** and **H**. The presence of the α-ketol group was supported by a positive colour reaction with alkaline triphenyltetrazolium. The elements were characterized by high resolution COSY NMR as follows. Element **A** consisted of one angular methyl group, a 2,2,4,4-tetrasubstituted cyclopentene ring, and a cyclohexanone ring with



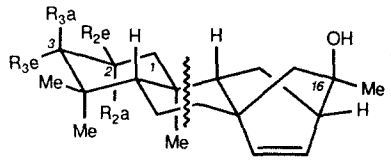
ent-BEYERANE FAMILY

	ELEMENTS	R_{2a} R_{2e}	R_{3a} R_{3e}	R_{12a} R_{12e}	
B- 1		H H	H H	H H	
B- 5	H' + A	OH	=O	=O	1,2-dehydro-
B- 6	I + A	H H	=O	=O	
B- 7	I' + A	=O	H H	=O	
B- 9	H + A	=O	H OH	=O	
B'-11	H'' + A	OH H	=O	=O	1-nor-
B-14	J + A	H H	H OH	=O	
B-18	H + B	=O	H OH	H OH	
B-20	H + B'	=O	H OH	OH H	
B-22	K + A	H OH	H OH	=O	



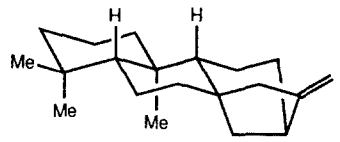
ent-PIMARANE FAMILY

	ELEMENTS	R_{2a} R_{2e}	R_{3a} R_{3e}	R_{12a} R_{12e}	
P- 2		H H	H H	H H	
P- 4	I + C	H H	=O	=O	
P- 8	H + E	=O	H OH	=O	9(11)-dehydro-8β,14β-O-
P-10	H + E'	=O	H OH	=O	9(11)-dehydro-8α,14α-O-
P-12	J + C	H H	H OH	=O	
P-13	I + D	H H	=O	H OH	
P-15	H + F	=O	H OH	=O	7,9(11)-tetradehydro-14β-OH
P-17	H + D	=O	H OH	H OH	
P-21	J + D'	H H	H OH	OH H	



ent-ATISANE FAMILY

	ELEMENTS	R_{2a} R_{2e}	R_{3a} R_{3e}
A-16	I + G	H H	=O
A-19	H + G	=O	H OH



ent-KAURANE FAMILY

K- 3

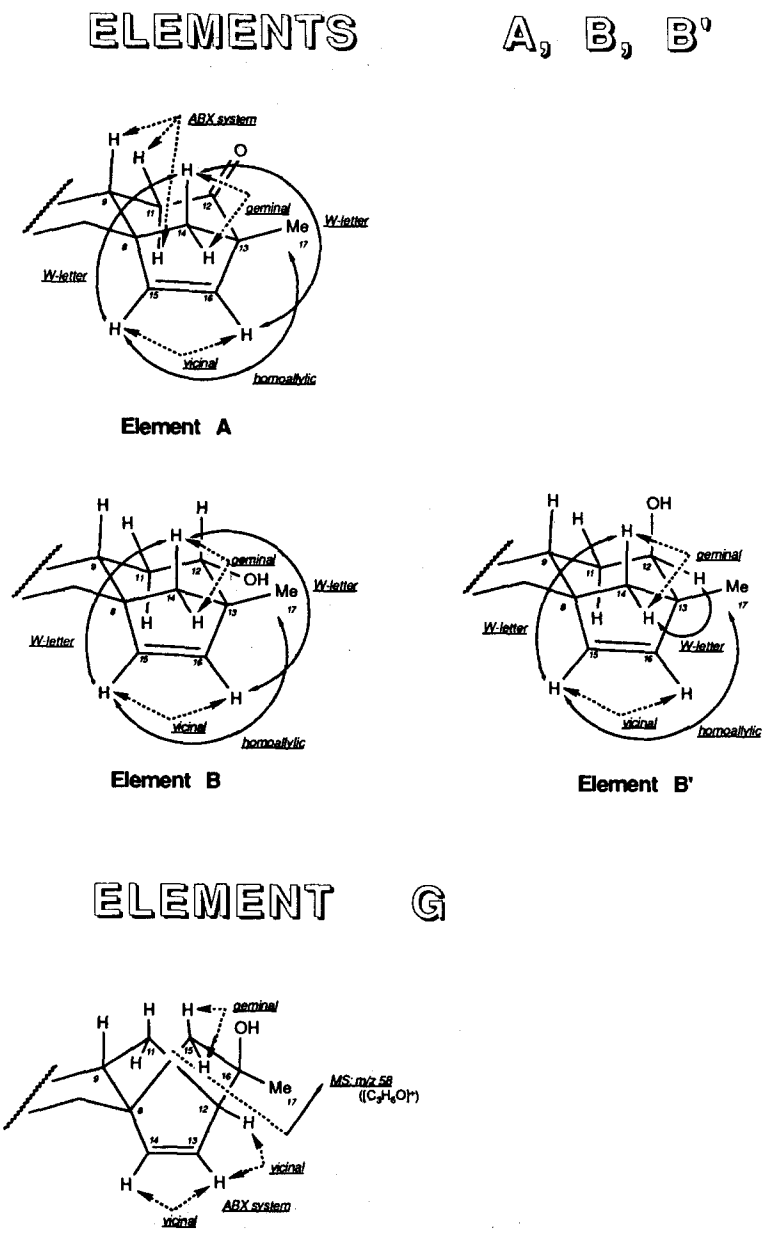
a C^9H–$C^{11}H_2$–$C^{12}O$ moiety (ABX system) and is characterized by the presence of two *W*-type long-range couplings between H-14a/H-15 and H-14a/H-16, and a homoallylic coupling between H-15 and H-17 (13-Me). Element **H** was characterized by the following; three tertiary methyl groups are present as geminal and angular methyls on the other cyclohexanone ring with a C^1H_2–C^2O–C^3H (OH) moiety (α-ketol); the presence of a 1,3-diaxial coupling between H-3a and H-1a, and a *W*-type coupling between H-1a and H-20 (10-Me) were observed in the COSY spectrum. Another possibility of the ketone group at C-7, that is the presence of an ABX system at C^5H–C^6H_2–C^7O, was excluded because of NOEs observed between H-3a and H-5, and H-1a and H-5, H-5 corresponding to the C^5H–C^6H_2–C^7H_2 spin system. The stereochemistry of **B-9** was determined by NOEs between H-15 and H-20 (10-Me), and H-11a and H-20 (10-Me), moreover supported by the co-occurrence of **B-1** which is the mother skeleton of this family. The structure of **B-9** was established to be *ent*-3β-hydroxybeyer-15-ene-2,12-dione, and this structure had been already assigned to a major diterpene component of the heartwood of *Androstachys johnsonii* Prain (Euphorbiaceae) [3]. Although ^1H and ^{13}C NMR data of **B-9** coincide with those reported [4] the optical rotation value of our compound ($[\alpha]_D^{23} -73.9°$) differs from that reported ($[\alpha]_D -329°$).

The mass and IR spectra suggested that **B-5**, $C_{20}H_{26}O_3$, is a dehydro-derivative of **B-9** with one conjugated and one unconjugated ketone and one hydroxyl group. The ^1H NMR and UV spectra indicated **B-5** to be the known corresponding diosphenol, *ent*-2-hydroxybeyera-1, 15-diene-3, 12-dione (elements **A** and **H'**), previously isolated from the heartwood of *A. johnsonii* as a minor component [3]. Oxidation of **B-9** with Bi_2O_3 gave a diosphenol, which was identical to **B-5**.

The spectral data indicated that **B-6** and **B-7** have the same molecular formula, $C_{20}H_{28}O_2$, with two ketone groups and the presence of element **A**. The location of the second ketone group was determined to be at C-3 on ring A (element **I**) in **B-6** and at C-2 (element **I'**) in **B-7** by their COSY spectra. Element **I** was demonstrated by the presence of an ethylene group adjacent to a ketone group (C^1H_2–C^2H_2–C^3O moiety) and a *W*-type coupling between H-1a and H-20 (10-Me), while the presence of two isolated active methylene protons (C^1H_2–C^2O–C^3H_2 moiety) and two *W*-type couplings between H-1a/H-20 (10-Me) and H-3a/H-19(4a-Me) was found in element **I'**. Therefore, **B-6** (elements **A** and **I**) is *ent*-beyer-15-ene-3,12-dione and **B-7** (elements **A** and **I'**) is *ent*-beyer-15-ene-2, 12-dione. *ent*-Beyer-15-ene-3, 12-dione was also isolated as its 2-benzylidene derivative from the hexane extract of *A. johnsonii* [5].

Although the MS data of both **B'-11** and **B-14** showed the same molecular peak at *m/z* 302, the molecular formula of **B-14** was $C_{20}H_{30}O_2$, while that of **B'-11** was $C_{19}H_{26}O_3$; IR spectra showed the presence of one hydroxyl and one ketone group in **B-14**, and one hydroxyl and two ketone groups in **B'-11**. The presence of element A in both **B'-11** and **B-14** was apparent from their COSY spectra.

The A ring portion in **B-14**, element **J**, was determined to be a dihydro derivative of element **I** based on the presence of three tertiary methyl groups, a



C^1H_2–C^2H_2–C^3H(OH) spin system, and a *W*-type coupling between H-1a and H-20 (10-Me). The configuration of the hydroxyl group at C-3 was assigned to be equatorial because of the large coupling constant of the hydroxy methine proton. On the other hand, the A ring moiety in norditerpene **B'-11**, element **H''**, consisted of three tertiary methyl groups and a cyclopentanone ring with an α-ketol group. The positive colour reaction supported the presence of the α-ketol, whereas the presence of two *W*-type couplings between H-1a and H-20 (10-Me), and H-5 and H-20 (10-Me) suggested that the hydroxyl group was located at C-1 and equatorial, thus placing the ketone group at C-2. Consequently, **B'-11** (elements **A** and **H''**) is *ent*-1β-hydroxy-3-norbeyer-15-ene-2,12-dione and **B-14** (elements **A** and **J**) is *ent*-3β-hydroxybeyer-15-en-12-one.

The three components **B-18, B-20,** and **B-22** have the same molecular formula $C_{20}H_{30}O_3$ with two secondary hydroxyls and one ketone group. The COSY spectra suggested both **B-18** and **B-20** to have element **H**. The C/D ring portions in **B-18** and **B-20** were also suggested to have the same planar structures and were assigned as dihydro derivatives of element **A**, elements **B** and **B'** respectively, by tracing the spin systems there. The presence of the hydroxyl group at C-12 was in agreement with their ^{13}CNMR spectra when compared with that of element **A**. The configuration at C-12 was determined from the *J* value of the hydroxy methine protons, i.e. the

H-12 with a large coupling constant in **B-18** is β-axial (element **B**), while the H-12 with a small J value in **B-20** is α-equatorial (element **B'**). Thus, **B-18** (elements **B** and **H**) is *ent*-3β,12β-dihydroxybeyer-15-en-2-one and **B-20** (elements **B'** and **H**) is *ent*-3β-12α-dihydroxybeyer-15-en-2-one.

In contrast to the above two compounds, the spectral data of **B-22** showed the presence of element **A** and a C^1H_2–$C^2H(OH)$–$C^3H(OH)$ moeity in the **A** ring (element **K**). The location of these hydroxyl groups was determined based on the *W*-type coupling between H-1 and H-20 (10-Me), and large *trans* coupling constants between H-1a and H-2a, and H-2a and H-3a suggested both hydroxyls to be equatorial. Thus, **B-22** (elements **A** and **K**) is *ent*-2α, 3β-dihydroxybeyer-15-en-12-one.

ent-*Pimarane family*

The second group of DSM isolated from cassava was the *ent*-pimarane family comprising **P-4**,**-8**,**-10**,**-12**,**-13**, **-15**,**-17**, and **-21** along with the known (−)-*ent*-pimara-8 (14), 15-diene (**P-2**). All compounds of this family are also characterized by the presence of the typical pimaradiene type four singlet protons as shown in **P-2**, i.e. three olefinic protons constituting an AMX system arising from the vinyl group at C-13 and the singlet methine proton at C-14, either olefinic or oxygen-bearing, constituting elements **C**, **D**, **D'**, **E**, **E'**, and **F**.

The spectral data indicated that **P-4** has a molecular formula $C_{20}H_{28}O_2$ with two ketone groups. The COSY spectrum suggested that **P-4** is built up from elements **I** and **C**. The C ring portion (element **C**) was shown to have the typical pimaradiene type four olefinic protons, an angular methyl and a C^9H–$C^{11}H_2$–$C^{12}O$ moiety (ABX system). The position of the ketone group at C-12 was determined by the presence of an allylic coupling between H-9/H-14, H-9 corresponding to X of the ABX system. Thus **P-4** is determined to be *ent*-pimara-8(14),15-diene-3,12-dione based on the co-occurrence of mother component **P-2**.

Yucalexins **P-12** and **P-13** have the same molecular formula $C_{20}H_{30}O_2$ with one secondary hydroxyl and one ketone group. The COSY spectra indicated that both are dihydro derivatives of **P-4**; **P-12** is made up of elements **J** and **C** and **P-13** has elements **I** and **D**. Element **D** (the C ring portion of **P-13**) was suggested to be a hydroxy derivative in place of the ketone group at C-12 of element **C** based on the presence of a C^9H–$C^{11}H_2$–$C^{12}H(OH)$ spin system. The equatorial hydroxyl group was determined by the large coupling constant of H-12. Thus, **P-12** is *ent*-3β-hydroxypimara-8(14),15-dien-12-one and **P-13** is *ent*-12β-hydroxypimara-8(14),15-dien-3-one.

The spectral data showed **P-21** to have the molecular formula $C_{20}H_{32}O_2$ with two secondary hydroxyl groups and to be a dihydro derivative of **P-12** or **P-13**. The COSY spectrum showed the presence of elements **J** and **D'** which is the 12-epimer of element **D**. The axial configuration of the hydroxyl group at C-12 in element **D'** was in accordance with the small coupling constants of H-12. This resulted in downfield shifts of the signals due to H-12 and the three protons of 13-vinyl group compared to those in **P-13** with element **D**, while H-17(16-Me) and H-14 were shifted upfield. Thus, **P-21** is *ent*-3β,12α-dihydroxypimara-8(14),15-diene.

The spectral data also indicated that **P-17** has the molecular formula $C_{20}H_{30}O_3$ with elements **H** and **D**, that is, **P-17** is *ent*-3β,12β-dihydroxypimara-8(14),15-dien-3-one.

The mass spectra of yucalexins **P-8**, **P-10** and **P-15** showed the same molecular formula $C_{20}H_{26}O_4$. Inspection of the spectral data revealed that they have element **H**, and in their B/C ring moieties both **P-8** and **P-10** have the same planar structures with one α,β-unsaturated ketone, one 1,2-epoxy ring, and one angular methyl and one vinyl group, while **P-15** has one $\alpha\beta$,$\gamma\delta$-unsaturated ketone and one secondary hydroxyl. From the ^1H chemical shifts and other data, **P-8** and **P-10** were shown to be 8,14-epoxides of *ent*-3β-hydroxypimara-8(14),9(11),15-triene-2,12-dione. The configuration of the epoxy ring was assigned as β (*trans* to the vinyl group) in **P-8**, element **E**, and in **P-10** (element **E'**) as follows. The signal due to H-17(13-Me) in element **E** was shifted downfield from that in element **E'** by the anisotropic effect of the epoxy ring. On the contrary, the signals due to the vinyl group's three protons in element **E** were shifted upfield from those in element **E'** for the same reasons. In the ^{13}C NMR spectra, the signals due to C-5, C-6, C-7, C-10 and C-17 (13-Me) of **P-8** were also shifted upfield from those of **P-10**, since 13-Me and the B ring of **P-8** were sterically affected by the tension of the expoxy ring. Thus, **P-8** (elements **E** and **H**) is *ent*-8α,14α-epoxy-3β-hydroxypimara-9(11),15-diene-2,12-dione and **P-10** (elements **E'** and **H**) is *ent*-8β,14β-epoxy-3β-hydroxypimara-9(11),15-diene-2,12-dione.

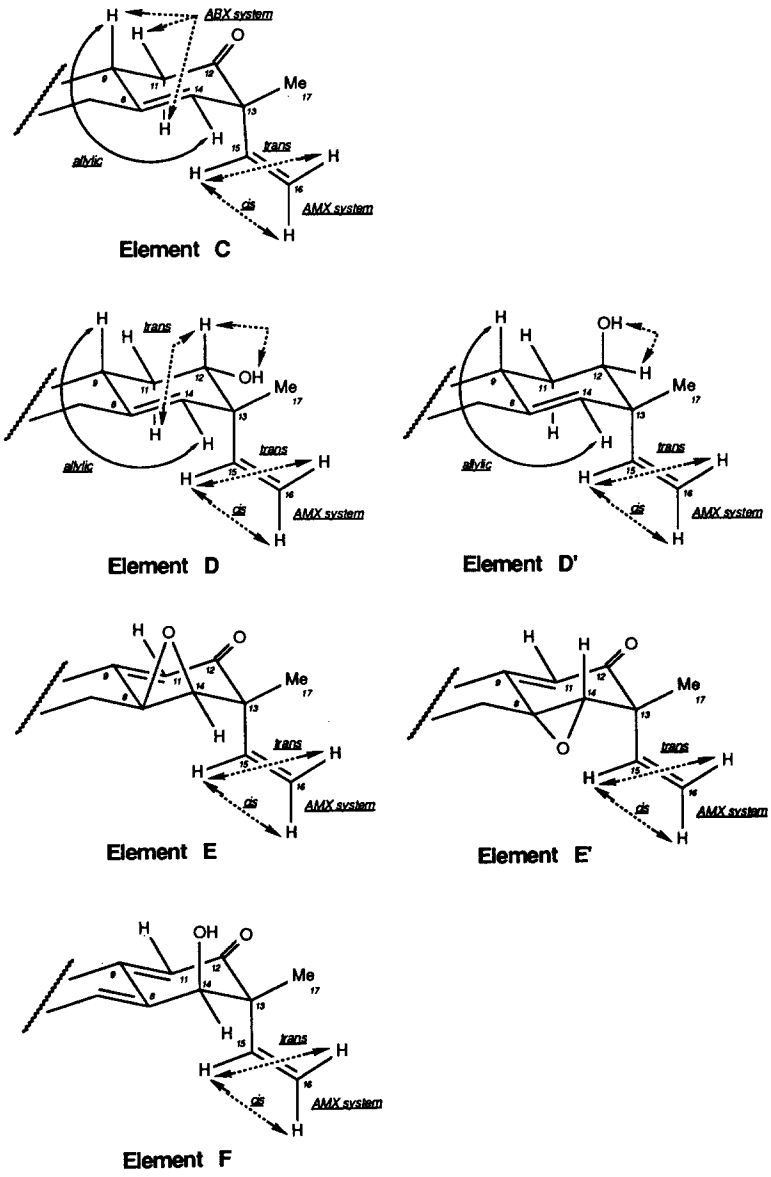
The structure of **P-15** was established by comparing its ^1H and ^{13}C NMR spectra with that of **P-8** and **P-10**. The configuration of the secondary hydroxyl group at C-14 was assumed to be β axial by biogenetic considerations, i.e. it was probable that **P-15** was derived from the major component **P-8** by an epoxy ring cleavage. Thus, **P-15** (elements **F** and **H**) is *ent*-3β,14α-hydroxypimara-7,9(11),15-triene-2,12-dione.

ent-*Atisane family*

The third group of DSM in injured cassava roots is the *ent*-atisane family comprising **A-16** and **A-19** which is the third major DSM constituent. These two compounds possess the three protons characteristic of the atis-13-ene type, i.e. two olefin protons and one bridge head methine proton due to the $C^{14}H{=}C^{13}H$–$C^{12}H$ moiety (ABX system).

The $[M]^+$ of **A-19** was not observed in the EI mass spectrum, but the CI spectrum indicated that it has the molecular formula $C_{20}H_{30}O_3$ ($[M+H]^+$ m/z 319, 100%). The spectral data indicated that **A-19** has two secondary hydroxyls and one ketone group; the A ring portion is element **H**. The presence of a $C^{15}H_2$–$C^{16}(OH)$–$C^{17}H_3$ moiety in the C/D ring portion of atis-13-ene skeleton, element **G**, was indicated by the base peak observed in the EI mass spectrum [m/z 260 $[M-58]^+$, 100%]. NOE experiments revealed that the configuration of the tertiary hydroxyl group at C-16 is *trans* to C-13 and C-14, because of the NOE's between H-13 and H-17 (16-Me), and H-14 and H-17 (16-Me). Thus, **A-19** is *ent*-3β,16α-dihydroxyatis-13-en-2-one.

In the EI mass spectrum of **A-16** the $[M]^+$ m/z 302 ($C_{20}H_{30}O_2$) was not observed, the base peak being $[M-MeC(OH)CH_2]^+$ (m/z 244). The COSY spectrum indicated the presence of elements **I** and **G**. Thus, **A-16** is *ent*-16α-hydroxyatis-13-en-3-one. Piaccnza *et al.* also isolated **A-16** as its 2-benzylidene derivative from the heartwood of *A. johnsonii* [5].



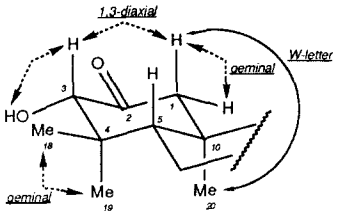
ent-*Kaurane family*

ent-Kaurene (**K-3**) was found as the only component belonging to this family in injured cassava roots.

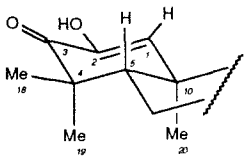
The cassava DSM belong to the basic types of the tri- (**P**) and tetracyclic diterpenes (**B, A** and **K**). The biogenetic pathways from geranylgeranyl pyrophosphate to the tri- and tetracyclic diterpenes are well known [6]. The co-occurrence of these four types of diterpenes, especially *ent*-beyerane and *ent*-atisane type compounds in one species of plant is well documented. The co-occurrence of diterpenes with variation among ketone (element **I**), α-ketol (element **H**), and diosphenol (element **H'**) was also found in the cassava root tissues (**B-6/B-9/B-5,P-4,P-13/P-17/—**, and **A-16/A-19/–**), in analogy with that in the heartwood of *Androstachys johnsonii* [3] and *Spirostachys africana* [7]. The interrelationship of these substances has been established by chemical transformations [3]. Yucalexins **B-18** and **B-20** appear to be intermediates in the biogenetic pathway of the third major component **A-19** from the major component **B-9**. This pathway was also supported by chemical transformations consisting of *p*-tosylhydrazine treatment followed reductive rearrangement [8]. The second major component **P-8** and its 8,14-isomer **P-10** were presumably derived from **P-17** or its 12-keto derivative yet to be detected by dehydrogenation and epoxidation, further ring cleavage yielding **P-15** from **P-8**.

A number of studies have been performed on phytoalexins of major important food crops [9], e.g. on potato tubers (Solanaceae), sweet potato roots (Convolvulaceae), rice leaves (Gramineae) and a few on cassava roots

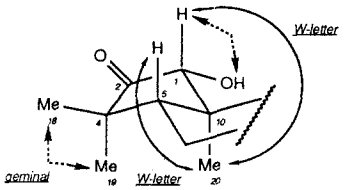
ELEMENTS H, H', H'', I, I', J, K



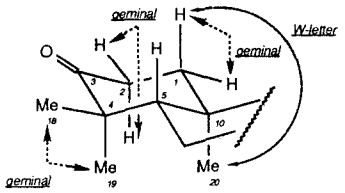
Element H



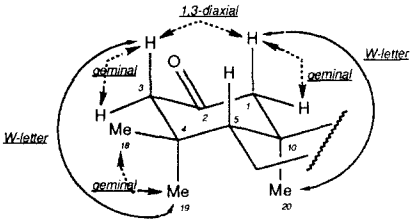
Element H'



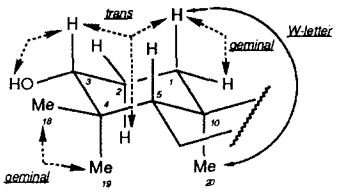
Element H''



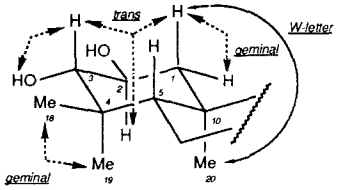
Element I



Element I'



Element J



Element K

(Euphorbiaceae). More than 20 sesquiterpenic phytoalexins have been isolated from sweet potato roots, potato tubers, and tobacco leaves damaged in response to stress or infection. In our present study, a total of 22 DSM have been isolated from the damaged cassava root tissues.

Although the sesquiterpenic stress metabolites are numerous, only very few have been reported as diterpenic phytoalexins or stress compounds. Namely, casbene is a hydrocarbon phytoalexin isolated from castor bean (*Ricinus communis*; Euphorbiaceae) [10], and momilactones A and B are diterpenic lactones isolated from rice (*Oryza sativa*) leaves [11]. Recently, three diterpenic phytoalexins with the (+)−sandaracopimaradiene skeleton (oryzalexins A, B, and C) were isolated from rice leaves infected with a fungus [12]. Our present results constitute the fourth example of DSM. The expected antibiotic activity of each component has not been determined, but further work is in progress to investigate their biological activities.

EXPERIMENTAL

General. Optical rotations were measured in $CHCl_3$. GC/MS measurements were performed with a glass column (3 mm × 1 m) packed with OV-17. NMR spectra were recorded at 25 MHz for ^{13}C, 360 MHz for ^1H in $CDCl_3$ with TMS as int sd.

Materials and separations. Fr. cassava roots (cv 'Golden Yellow' grown in Baybay, Leyte, Phillipines) were cut vertically into slices 1 cm thick and incubated for 7 days at 25–30°, r.h. 75–95%. Damaged parts of slices were severed, cut into small pieces (5 kg) and air-dried for 2 days under the same conditions to give chips of the damaged root tissues (2.6 kg). These chips were pulverized with a blender and extracted with MeOH. The crude MeOH ext (36.2 g) was dispersed in H_2O and the aq. suspension extd with EtOAc. The EtOAc ext (5.1 g) was fractionated by flash chromatography on silica gel to give two fractions, a $CHCl_3$ and a 10% MeOH–$CHCl_3$ one (the more-polar fraction **4**, 2.11 g). The first $CHCl_3$ fraction was further divided by silica gel CC into three sub-fractions; the non-polar fraction **1** (eluted with hexane, 36.9 mg), the least-polar fraction **2** ($CHCl_3$, 1.66 g), and the less-polar fraction **3** (Et_2O, 1.20 g). The DSM were present in the fractions **1**, **3**, and **4**. By repeated CC using lobar columns (LiChroprep Si 60, Size A and B, Merck) and finally by normal (Develosil SI 60, ϕ 6 mm × 25 cm, Nomura Chemicals) or reverse (μBondapak™ C18, ϕ 6 mm × 25 cm, Waters) phase HPLC, 22 components were isolated and purified.

Yucalexin **P-4** [*ent*-pimara-8 (14),15-diene-3,12-dione, 1.1 mg, Fr. 3]. IR $\nu_{max}cm^{-1}$: 1715 (C=O). EIMS 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (74), 285 (5), 243 (5), 173 (9), 119 (21), 105 (38), 95 (100), 91 (25), 79 (14), 67 (13), 55 (17), 41 (25). ^1H NMR: see Table 2.

Yucalexin **B-5** [*ent*-2-hydroxybeyera-1,15-diene-3,12-dione, 1.7 mg, Fr. 3]. IR $\nu_{max}cm^{-1}$: 3420 (OH), 1710 (C=O); 1670 (C=C–C=O). EIMS 70 eV, *m/z* (rel. int.): 314 $[M]^+$ (89), 299 (26), 271 (30), 215 (12), 152 (41), 135 (51), 119 (84), 105 (60), 93 (100), 91 (91), 76 (60), 41 (48). ^1H NMR: see Table 1.

Yucalexin **B-6** [*ent*-beyer-15-ene-3,12-dione, 6.6 mg, Fr. 3]. IR $\nu_{max}cm^{-1}$: 1705 (C=O). EIMS 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (100), 272 (26), 257 (10), 173 (11), 145 (15), 133 (17), 119 (34), 106 (61), 93 (79), 91 (51), 77 (36), 67 (23), 55 (24), 41 (39). ^1H NMR: see Table 1.

Yucalexin **B-7** [*ent*-beyer-1,15-en-2,12-dione, 3.4 mg, Fr. 3]. IR $\nu_{max}cm^{-1}$: 1710 (C=O). EIMS 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (100), 285 (6), 272 (5), 257 (10), 243 (15), 173 (17), 120 (31), 106 (53), 93 (90), 77 (35), 67 (16), 55 (18), 41 (33). ^1H NMR: see Table 1.

Yucalexin **P-8** [*ent*-8α,14α-epoxy-3β-hydroxypimara-9(11), 15-diene-2,12-dione, 18.0 mg. Fr. 3]. IR $\nu_{max}cm^{-1}$: 3470 (OH), 1720 (C=O), 1675 (C=C–C=O). UV λ_{max}^{MeOH} nm (log ε): 262 (3.649). EIMS 70 eV, *m/z* (rel. int.): 330 $[M]^+$ (43), 315 (35), 302 (23), 287 (52), 243 (25), 215 (30), 187 (34), 176 (31), 162 (59), 135 (45), 119 (51), 105 (71), 91 (91), 77 (58), 67 (43), 55 (52), 41 (100). ^1H NMR: see Table 2. ^{13}C NMR: δ 15.6 (*q*, C-20), 16.8 (*t*, C-6), 19.4 (*q*, C-17), 23.1 (*q*, C-19), 25.6 (*t*, C-7), 27.7 (*q*, C-18), 43.6 (*d*, C-5), 44.2 (*s*, C-10), 44.8 (*s*, C-4), 49.6 (*s*, C-13), 51.0 (*t*, C-1), 55.5 (*s*, C-8), 64.8 (*d*, $^1J_{CH}$=179.5 Hz, C-14), 81.9 (*d*, C-3), 116.7 (*t*, C-16), 121.1 (*d*, C-11), 138.4 (*d*, C-15), 167.9 (*s*, C-9), 196.8 (*s*, C-12), 208.7 (*s*, C-2).

Yucalexin **B-9** [*ent*-3β-hydroxybeyer-15-ene-2, 12-dione, 47.8 mg, Fr. 3] $[\alpha]_D^{23}$ −73.9° ($CHCl_3$, *c* 0.83). IR $\nu_{max}cm^{-1}$: 3480

Table 1. ^1H NMR data of *ent*-beyerane family

Name	Element		H-1	H-2	H-3	H-5	H-18	H-19	H-20
B-5	H′	ax	6.11 *s*	5.98 *s* (OH)		—		1.09 *s*	1.15 *s*
		eq					1.13 *s*		
B-6	1	ax	1.38 *ddd*	2.57 *ddd*		1.37 *dd*		1.07 *s*	0.95 *s*
		eq	1.76 *ddd*	2.33 *ddd*			1.11 *s*		
			J 1a, 1e = 13, 1a, 2a = 12, 1a, 2e = 6, 1e, 2a = 7, 1e, 2e = 3, 2a, 2e = 15.5 Hz						
			0.95/1.38 (*W*-letter)						
B-7	I′	ax	2.00 *dd*		2.32 *dd*	1.94 *dd*		0.90 *s*	0.78 *s*
		eq	2.14 *d*		2.16 *d*		1.09 *s*		
			J 1a, 1e = 12.5, 3a, 3e = 13 Hz; 1a, 3a = 2 Hz (1, 3-diaxial)						
			0.78/2.00, 0.90/2.32 (*W*-letter)						
B-9	H	ax	2.09 *dd*		3.90 *dd*	1.57 *dd*		0.73 *s*	0.76 *s*
		eq	2.31 *d*		3.40 *d* (OH)		1.21 *s*		
			J 1a, 1e = 11.5, 3a, 3OH = 6 Hz; 1a, 3a = 2 Hz (1,3-diaxial)						
			0.76/2.09 (*W*-letter)						
B′-11	H″	ax	3.93 *d*			—		1.03 *s*	0.66 *s*
		eq	2.57 *d* (OH)				1.12 *s*		
			J 1a, 1e = 3 Hz.						
			0.66/3.93 (*W*-letter)						
B-14	J	ax	0.99 *ddd*	1.49 *dddd*	3.21 *dd*	0.86 *dd*		0.80 *s*	0.77 *s*
		eq	1.48 *ddd*	1.61 *dddd*	−(OH)		1.01 *s*		
			J 3a, 2a = 11.5, 3a, 2e = 5 Hz						
			0.77/0.99 (*W*-letter)						
B-18	H	ax	2.13 *dd*		3.90 *ddd*	—		0.70 *s*	0.75 *s*
		eq	2.45 *d*		−(OH)		1.19 *s*		
			J 1a, 1e = 12Hz; 1a, 3a = 1 Hz (1, 3-diaxial)						
			0.75/2.13 (*W*-letter)						
B-20	H	ax	2.15 *dd*		3.91 *dd*	—		0.70 *s*	0.67 *s*
		eq	2.39 *d*		3.40 *d* (OH)		1.20 *s*		
			J 1a, 1e = 12, 3a, 3e = 4 Hz; 1a, 3a = 1 Hz (1,3-dialxial)						
			0.67/2.15 (*W*-letter)						
B-22	K	ax	0.93 *dd*	3.65 *dddd*	3.00 *dd*	0.97 *dd*		0.86 *s*	0.85 *s*
		eq	1.82 *dd*	2.05 *d* (OH)	2.19 *d* (OH)		1.11 *s*		
			J 1a, 1e = 12, 1a, 2a = 12, 1e, 2a = 4.5, 2a, 2OH = 3.5, 2a, 3a = 9.5, 3a, 3OH = 4 Hz						
			0.85/0.93 (*W*-letter)						

Table 1. (*Continued*)

Name	Element		H-9	H-11	H-12	H-14	H-15	H-16	H-17
B-5	**A**	ax	1.91 *dd*	2.60 *dd*		1.67 *d*	6.07 *d*	5.70 *d*	1.26 *s*
		eq		2.50 *dd*		1.95 *d*			
			J 9, 11a = 11, 9, 11e = 6.5, 11a, 11e = 16.5 Hz (ABX); 14a, 14e = 11, 15, 16 = 5.5 Hz (AB *q*)						
B-6	**A**	ax	1.64 *dd*	2.48 *dd*		1.65 *d*	6.10 *d*	5.67 *d*	1.11 *s*
		eq		2.31 *dd*		1.94 *d*			
			J 9, 11a = 10.5, 9, 11e = 6, 11a, 11e = 16.5 Hz (ABX); 14a, 14e = 10.5, 15, 16 = 5.5 Hz (AB *q*)						
			1.65/6.10 (*W*-letter); 1.11/6.10 (homoallylic)						
B-7	**A**	ax	1.87 *dd*	2.41 *dd*		1.65 *d*	6.02 *d*	5.63 *d*	1.11 *s*
		eq		2.21 *dd*		1.94 *d*			
			J 9, 11a = 10.5, 9, 11e = 6.5, 11a, 11e = 16.5 Hz (ABX); 14a, 14e = 11, 15, 16 = 6 Hz (AB *q*)						
			1.65/6.02 (*W*-letter); 1.11/6.02 (homoallylic)						
B-9	**A**	ax	1.91 *dd*	2.42 *dd*		1.66 *d*	6.02 *d*	5.66 *d*	1.12 *s*
		eq		2.21 *dd*		1.97 *d*			
			J 9, 11a = 11.5, 9, 11e = 7, 11a, 11e = 17 Hz (ABX); 14a, 14e = 11.5, 15, 16 = 6 Hz (AB *q*)						
			1.66/5.66 (*W*-letter); 1.12/6.02 (homoallylic)						
B′-11	**A**	ax	2.04 *dd*	2.68 *dd*		1.74 *d*	6.06 *d*	5.60 *d*	1.10 *s*
		eq		2.50 *dd*		1.99 *d*			
			J 9, 11a = 11, 9, 11e = 6, 11a, 11e = 17 Hz (ABX); 14a, 14e = 11, 15, 16 = 5.5 Hz (AB *q*)						
			1.74/6.03 (*W*-letter); 1.10/6.03 (homoallylic)						
B-14	**A**	ax	1.56 *dd*	2.40 *dd*		1.60 *d*	6.03 *d*	5.72 *d*	1.10 *s*
		eq		2.27 *dd*		1.89 *d*			
			J 9, 11a = 11, 9, 11e = 7, 11a, 11e = 17 Hz (ABX); 14a, 14e = 11, 15, 16 = 6 Hz (AB *q*)						
			1.60/6.06 (*W*-letter); 1.10/6.06 (homoallylic)						
B-18	**B**	ax	1.39 *dd*	1.81 *ddd*	3.51 *dd*	1.06 *d*	5.78 *d*	5.62 *d*	1.13 *s*
		eq		1.14 *ddd*	−(OH)	1.58 *d*			
			J 9, 11a = 12, 9, 11e = 5, 11a, 11e = 11, 11a, 12a = 9, 11e, 12a, = 6 Hz; 14a, 14e = 9, 15, 16 = 5.5 Hz (AB *q*)						
			1.06/5.62, 1.06/5.78 (*W*-letter); 1.13/5.78 (homoallylic)						
B-20	**B′**	ax	2.35 *dd*	1.66 *ddd*	3.41 *d* (OH)	1.29 *d*	5.70 *d*	5.61 *d*	1.08 *s*
		aq		1.46 *ddd*	3.71 *ddd*	1.78 *d*			
			J 11a, 12e = 1.5, 11e, 12e = 1.5, 12e, 12OH = 3 Hz; 14a, 14e = 11, 15, 16 = 6 Hz (AB *q*)						
			1.29/5.70, 1.78/3.71 (*W*-letter); 1.08/5.70 (homoallylic)						
B-22	**A**	ax	1.63 *dd*	2.43 *dd*		1.62 *d*	6.02 *d*	5.62 *d*	1.10 *s*
		eq		2.30 *dd*		1.91 *d*			
			J 9, 11a = 10.5, 9, 11e = 6.5, 11a, 11e = 17 Hz (ABX); 14a, 14e = 11, 15, 16 = 5.5 Hz (AB *q*)						
			1.62/6.02 (*W*-letter); 1.10/6.02 (homoallylic)						

(OH), 1710 (C=O). HRMS: M^+ 316.2045; $C_{20}H_{28}O_3$ requires 316.2038. EIMS 70 eV, *m/z* (rel. int.): 316 $[M]^+$ (50), 243 (26), 201 (33), 119 (31), 105 (50), 93 (100), 91 (66), 77 (50), 41 (52). ^{1}H NMR: see Table 1. ^{13}C NMR: δ 14.9 (*q*, C-20), 16.5 (*q*, C-6), 17.1 (*q*, C-17), 20.0 (*t*, C-19), 29.3 (*q*, C-18), 35.7 (*t*, C-7), 36.1 (*t*, C-11), 43.5 (*s*, C-10), 45.6 (*s*, C-4), 49.5 (*s*, C-8), 51.0 (*t*, C-1), 53.8 (*d*, C-5), 54.7 (*d*, C-9), 57.3 (*s*, C-13), 58.0 (*t*, C-14), 82.7 (*d*, C-3), 136.9 (*d*, C-16), 138.5 (*d*, C-15), 210.0 (*s*, C-12), 210.8 (*s*, C-2).

Yucalexin **P-10** [*ent*-8β,14β-epoxy-3β-hydroxypimara-9(11),15-diene-2,12-dione, 5.6 mg, Fr. **3**]. IR ν_{max}cm^{-1}: 3480 (OH), 1720 (C=O), 1665 (C=C–C=O). UV λ_{max}^{MeOH} nm (log ε): 238 (3.780). EIMS 70 eV, *m/z* (rel. int.): 330 $[M]^+$ (17), 315 (17), 302 (26), 287 (65), 215 (23), 187 (25), 162 (100), 149 (31), 135 (31), 120 (29), 105 (42), 80 (47), 67 (44), 55 (51), 41 (99). ^{1}H NMR: see Table 2. ^{13}C NMR: δ 16.6 (*q*, C-20), 19.0 (*t*, C-6), 23.4 (*q*, C-17), 23.6 (*q*, C-19), 29.0 (*q*, C-18), 33.0 (*t*, C-7), 45.1 (*s*, C-4), 46.1 (*s*, C-10), 48.2 (*s*, C-13), 48.8 (*d*, C-5), 49.1 (*t*, C-1), 53.1 (*s*, C-8), 64.3 (*d*, $^1J_{CH}$ = 179.5 Hz, C-14), 82.1 (*d*, C-3), 115.6 (*t*, C-16), 120.9 (*d*, C-11), 137.8 (*d*, C-15), 166.6 (*s*, C-9), 198:7 (*s*, C-12), 208.8 (*s*, C-2).

Yucalexin **B′-11** [*ent*-1β-hydroxy-3-norbeyer-15-ene-2,12-dione, 0.8 mg, Fr. **3**]. IR ν_{max}cm^{-1}: 3346 (OH), 1750 (cyclopentanone), 1710 (C=O). EIMS 70 eV, *m/z* (rel. int.): 302 $[M]^+$ (32), 274 (14), 202 (29), 173 (19), 159 (12), 105 (21), 93 (100), 91 (43), 77 (31), 69 (10), 55 (17), 41 (29). ^{1}H NMR: see Table 1.

Yucalexin **P-12** [*ent*-3β-hydroxypimara-8(14),15-dien-12-one, 5.5 mg, Fr. **3**]. IR ν_{max}cm^{-1}: 3290 (OH), 1715 (C=O). EIMS 70 eV, *m/z* (rel. int.): 302 $[M]^+$ (43), 284 (11), 269 (10), 173 (9), 135 (100), 119 (25), 105 (44), 95 (45), 91 (33), 79 (20), 69 (17), 55 (25), 41 (34). ^{1}H NMR: see Table 2.

Yucalexin **P-13** [*ent*-12β-hydroxypimara-8(14),15-dien-3-one, 1.0 mg, Fr. **3**]. IR ν_{max}cm^{-1}: 3400 (OH), 1705 (C=O). EIMS 70 eV, *m/z* (rel. int.): 302 $[M]^+$ (31), 284 (21), 269 (15), 173 (17), 151 (38), 134 (73), 133 (65), 123 (51), 119 (46), 105 (67), 91 (68), 81 (50), 67 (43), 55 (77), 41 (100). ^{1}H NMR: see Table 2.

Yucalexin **B-14** [*ent*-3β-hydroxybeyer-15-en-12-one, 3.1 mg, Fr. **3**]. IR ν_{max}cm^{-1}: 3330 (OH), 1710 (C=O). EIMS 70 eV, *m/z* (rel. int.): 302 $[M]^+$ (72), 274 (17), 175 (8), 149 (21), 135 (36), 121 (36), 106 (77), 93 (100), 77 (43), 67 (21), 55 (33), 41 (55),. ^{1}H NMR: see Table 1. ^{13}C NMR: δ 14.0 (*q*, C-20), 15.5 (*q*, C-19), 17.2 (*q*, C-17), 19.8 (*t*, C-6), 27.0 (*t*, C-2), 28.1 (*q*, C-18), 36.2 (*t*, C-7), 36.2 (*t*, C-11), 36.5 (*t*, C-1), 49.2 (*s*, C-8), 54.6 (*d*, C-9), 54.9 (*d*, C-5), 57.3 (*s*, C-13), 58.4 (*t*, C-14), 78.7 (*d*, C-3), 136.1 (*d*, C-16), 139.5 (*d*, C-15).

Yucalexin **P-15** [*ent*-3β,14α-dihydroxypimara-7,9(11),15-triene-2,12-dione, 4.2 mg, Fr. **4**]. IR ν_{max}cm^{-1}: 3485 (OH), 1720

Table 2. ^{1}H NMR data of *ent*-pimarane family

Name	Element		H-1	H-2	H-3	H-5	H-18	H-19	H-20
P-4	**I**	ax	1.47 *ddd*	2.63 *ddd*		1.50 *dd*		1.09 *s*	1.05 *s*
		eq	1.88 *ddd*	2.33 *ddd*			1.12 *s*		
			J 1a, 1e = 13, 1a, 2a = 11, 1a, 2e = 5, 1e, 2a = 5.5, 1e, 2e = 3, 2a, 2e = 17 Hz						
			1.05/1.47 (*W*-letter)						
P-8	**H**	ax	2.60 *dd*		4.01 *dd*	2.27 *dd*		0.83 *s*	1.23 *s*
		eq	2.78 *d*		3.39 *d*(OH)		1.22 *s*		
			J 1a, 1e = 12.5, 3a, 3OH = 5 Hz; 1a, 3a = 1.5 Hz (1, 3-diaxial)						
			1.23/2.60 (*W*-letter)						
P-10	**H**	ax	2.62 *dd*		3.99 *dd*	—		0.80 *s*	1.25 *s*
		eq	2.77 *d*		3.44 *d* (OH)		1.27 *s*		
			J 1a, 1e = 12.5, 3a, 3 OH = 5 Hz; 1a, 3a = 1.5 Hz (1, 3-diaxial)						
			1.25/2.62 (*W*-letter)						
P-12	**J**	ax	1.13 *ddd*	1.55 *dddd*	3.26 *ddd*	1.05 *dd*		0.84 *s*	0.84 *s*
		eq	1.10 *ddd*	1.67 *dddd*	−(OH)		1.03 *s*		
			J 1a, 1e = 13.5, 1a, 2a = ?, 1a, 2e = 3.5, 1e, 2a = 4, 1e, 2e = 3.5, 2a, 2e = 12.5						
			2a, 3a = 12, 2e, 3a = 3.5 Hz						
			0.84/1.13 (*W*-letter)						
P-13	**I**	ax	1.45 *ddd*	2.65 *ddd*		—		1.08 *s*	1.00 *s*
		eq	1.90 *ddd*	2.30 *ddd*			1.10 *s*		
			J 1a, 2a = 15, 1e, 2a = 6, 2a, 2e = 6 Hz						
			1.00/1.45 (*W*-letter)						
P-15	**H**	ax	2.66 *dd*		3.99 *dd*	2.09 *dd*		0.84 *s*	1.07 *s*
		eq	2.71 *d*		3.43 *d* (OH)		1.23 *s*		
			J 1a, 1e = 12, 3a, 3OH = 5 Hz; 1a, 3a = 1 Hz (1, 3-diaxial)						
			1.07/2.66 (*W*-letter)						
P-17	**H**	ax	2.29 *dd*		3.96 *dd*	1.69 *dd*		0.71 *s*	0.79 *s*
		eq	2.43 *d*		3.43 *d*(OH)		1.21 *s*		
			J 1a, 1e = 13, 3a, 3OH = 5 Hz; 1a, 3a = 2 Hz (1,3-diaxial)						
			0.79/2.29 (*W*-letter)						
P-21	**J**	ax	1.17 *ddd*	1.51 *dddd*	3.27 *m*	1.09 *dd*		0.83 *s*	0.76 *s*
		eq	1.13 *ddd*	1.62 *dddd*	1.40 *d*(OH)		1.02 *s*		
			J 3a, 3OH = 7.5, 3a, $W_{1/2}$ = 14 Hz						
			0.76/1.17 (*W*-letter)						

Name	Element		H-9	H-11	H-12	H-14	H-15	H-16	H-17
P-4	**C**	ax	2.28 *ddd*	2.62 *dd*		5.28 *dd*	5.85 *dd*	*cis* 5.17 *dd*	1.18 *s*
		eq		2.33 *dd*				*trans* 5.23 *dd*	
			J 9, 11a = 7, 9, 11e = 8, 11a, 11e = 10 Hz (ABX); 9, 14 = 1.5 Hz (allylic)						
			15, 16c = 10.5, 15, 16t = 17.5, 16c, 16t = 1 Hz (AMX)						
P-8	**E**			5.69 *s*		3.33 *s*	5.72 *dd*	*cis* 5.21 *d*	1.46 *s*
								trans 5.09 *d*	
			J 15, 16c = 11, 15, 16t = 17.5 Hz						
P-10	**E′**			5.70 *s*		3.16 *s*	6.09 *dd*	*cis* 5.28 *dd*	1.33 *s*
								trans 5.31 *dd*	
			J 15, 16c = 11, 15, 16t = 17.5, 16c, 16t = 1 Hz (AMX)						
P-12	**C**	ax	2.19 *ddd*	2.58 *dd*		5.20 *dd*	5.82 *dd*	*cis* 5.15 *dd*	1.16 *s*
		eq		2.27 *dd*				*trans* 5.21 *dd*	
			J 9, 11a = 8.5, 9, 11e = 7, 11a, 11e = 13.5 Hz (ABX); 9, 14 = 2 Hz (allylic);						
			15, 16c = 10, 15, 16t = 17, 16c, 16t = 1 Hz (AMX)						
P-13	**D**	ax	2.25 *ddd*	1.44 *ddd*	3.46 *m*	5.11 *dd*	5.92 *dd*	*cis* 5.11 *dd*	1.17 *s*
		eq		1.65 *ddd*	−(OH)			*trans* 5.22 *dd*	
			J 12a $W_{1/2}$ = 15 Hz; 9, 14 = 2 Hz (allylic); 15, 16c = 11, 15, 16t = 17.5, 16c, 16t = 2 Hz (AMX)						
P-15	**F**			5.64 *s*		4.35 *m*	5.93 *dd*	*cis* 5.47 *dd*	1.16 *s*
						1.88 *d* (OH)		*trans* 5.31 *dd*	
			J 14OH, 14e = 3 Hz; 15, 16c = 11, 15, 16t = 17.5, 16c, 16t = 1 Hz (AMX)						
P-17	**D**	ax	2.23 *ddd*	1.42 *ddd*	3.46 *ddd*	5.13 *dd*	5.88 *dd*	*cis* 5.21 *dd*	1.17 *s*
				1.61 *ddd*	3.40 *d* (OH)			*trans* 5.07 *dd*	
			J 9, 11a = 10, 9, 11e = 12, 11a, 12a = 12, 11e, 12a = 3.5, 12a, 12OH = 8 Hz;						
			15, 16c = 11, 15, 16t = 17.5, 16c, 16t = 11 Hz (AMX)						
P-21	**D′**	ax	1.84 *ddd*	1.41 *ddd*	1.31 *s* (OH)	5.07 *dd*	5.74 *dd*	*cis* 5.01 *dd*	1.09 *s*
		eq		1.68 *ddd*	3.64 *m*			*trans* 5.02 *dd*	
			J 9, 11a = 8, 9, 11e = 8, 11a, 11e = 11, 12e, $W_{1/2}$ = 10 Hz; 9/14 = 1.5 Hz (allylic);						
			15, 16c = 11, 15, 16t = 17, 16c, 16t = 1.5 Hz (AMX)						

(C=O), 1665 (C=C–C=O). UV λ_{max}^{MeOH} nm (log ε): 287 (3.894). ^{1}H NMR: see Table 2. ^{13}C NMR: δ 14.1 (*q*, C-20), 16.3 (*q*, C-19), 21.2 (*q*, C-17), 24.4 (*t*, C-6), 28.0 (*q*, C-18), 43.2 (*s*, C-10), 45.1 (*s*, C-4), 47.1 (*d*, C-5), 48.9 (*t*, C-1), 55.6 (*s*, C-13), 73.1 (*d*, C-14), 82.1 (*d*, C-3), 116.8 (*d*, C-7), 118.3 (*t*, C-16), 131.0 (*t*, C-8), 131.6 (*d*, C-11), 138.7 (*d*, C-15), 161.7 (*s*, C-9), 209.3 (*s*, C-3).

Yucalexin **A-16** [*ent*-16α-hydroxyatis-13-en-3-one, 0.4 mg, Fr. **4**]. IR ν_{max}cm^{-1}: 3445 (OH), 1700 (C=O). EIMS 70 eV, *m/z* (rel. int.): 244 [M − 58]$^+$ (100), 229 (20), 226 (16), 153 (21), 139 (38), 135 (36), 105 (21), 91 (70), 55 (24), 43 (57). ^{1}H NMR: see Table 3.

Yucalexin **P-17** [*ent*-3β,12β-dihydroxypimara-8 (14), 15-dien-2-one, 0.9 mg, Fr. **4**.]. IR ν_{max}cm^{-1}: 3435 (OH), 1715 (C=O). EIMS 70 eV, *m/z* (rel. int.): 318 [M]$^+$ (40), 300 (30), 285 (13), 274 (14), 227 (15), 185 (18), 167 (100), 159 (36), 134 (63), 119 (58), 107 (78), 105 (76), 91 (80), 79 (46), 69 (38), 55 (62), 41 (74). ^{1}H NMR: see Table 2.

Yucalexin **B-18** [*ent*-3β,12β-dihydroxybeyer-15-en-2-one, 5.0 mg, Fr. **4**.]. $[\alpha]_D^{26}$ −9.4° ($CHCl_3$, *c* 0.17). IR ν_{max}cm^{-1}: 3450 (OH), 1715 (C=O). EIMS 70 eV, *m/z* (rel. int.): 318 [M]$^+$ (52), 300 (18), 245 (24), 227 (27), 119 (32), 107 (100), 105 (55), 93 (59), 51 (35), 41 (39),. ^{1}H NMR: see Table 1. ^{13}C NMR: δ 15.8 (*q*, C-20), 16.6 (*q*, C-19), 20.0 (*t*, C-6), 20.5 (*q*, C-17), 29.4 (*q*, C-18), 30.4 (*t*, C-11), 35.9 (*t*, C-7), 43.7 (*s*, C-10), 45.7 (*s*, C-4), 48.5 (*s*, C-8), 48.7 (*s*, C-13), 51.6 (*t*, C-1), 52.1 (*d*, C-5), 54.2 (*d*, C-9), 58.3 (*t*, C-14), 73.9 (*d*, C-12), 82.7 (*d*, C-3), 134.7 (*d*, C-16), 136.4 (*d*, C-15), 210.7 (*s*, C-2).

Yucalexin **A-19** [*ent*-3β,16α-dihydroxyatis-13-en-2-one, 7.3 mg. , Fr. **4**]. $[\alpha]_D^{23}$ +8.28° ($CHCl_3$, *c* 0.35). IR ν_{max}cm^{-1}: 3440 (OH), 1710 (C=O). EIMS 70 eV, *m/z* (rel. int.): 260 [M − 58]$^+$ (100), 187 (10), 154 (17), 105 (34), 91 (46), 43 (15); CIMS: 319 [M + 1]$^+$, (100). ^{1}H NMR: see Table 3. ^{13}C NMR: δ 16.5 (*q*, C-19, C-20), 19.3 (*t*, C-6), 22.7 (*t*, C-11), 29.6 (*q*, C-18), 31.5 (*q*, C-17), 36.3 (*t*, C-7), 39.6 (*s*, C-10), 43.9 (*s*, C-8), 44.3 (*d*, C-12), 45.6 (*s*, C-4), 51.4 (*t*, C-1), 52.4 (*d*, C-9), 53.3 (*t*, C-15), 73.9 (*s*, C-16), 82.8 (*d*, C-3), 131.8 (*d*, C-13), 135.3 (*d*, C-14), 210.7 (*s*, C-2).

Yucalexin **B-20** [*ent*-3β,12α-dihydroxybeyer-15-ene-2-one, 2.6 mg, Fr. **4**]. IR ν_{max}cm^{-1}: 3425 (OH), 1715 (C=O). EIMS 70 eV *m/z* (rel. int.): 318 [M]$^+$ (66), 300 (19), 245 (20), 227 (23), 133 (26), 107 (100), 93 (43), 81 (34), 41 (38). ^{1}H NMR: see Table 1. ^{13}C NMR: δ 15.1 (*q*, C-20), 16.6 (*q*, C-19), 20.0 (*t*, C-6), 21.1 (*q*, C-17), 29.3 (*q*, C-18), 33.7 (*t*, C-11), 36.2 (*t*, C-7), 45.7 (*s*, C-4), 48.6 (*s*, C-13), 48.6 (*s*, C-10), 49.3 (*s*, C-8), 51.5 (*t*, C-1), 52.7 (*d*, C-5), 54.4 (*d*, C-9), 71.0 (*d*, C-12), 82.7 (*d*, C-3), 136.2 (*d*, C-16).

Yucalexin **P-21** [*ent*-3β,12α-dihydroxypimara-8 (14), 15-diene, 1.5 mg Fr. **4**]. IR ν_{max}cm^{-1}: 3460 (OH). EIMS 70 eV, *m/z* (rel. int.): 304 [M]$^+$ (2), 286 (21), 271 (23), 173 (4), 135 (100), 105 (23), 91 (20), 41 (19). ^{1}H NMR: see Table 2. ^{13}C NMR: δ 14.7 (*q*, C-20), 15.6 (*q*, C-19), 22.2 (*t*, C-6), 23.4 (*t*, C-1), 26.0 (*q*, C-17), 27.4 (*t*, C-2), 28.4 (*q*, C-18), 35.4 (*t*, C-10), 36.8 (*t*, C-11), 43.7 (*s*, C-7), 46.0 (*s*, C-4), 46.3 (*d*, C-5), 47.5 (*s*, C-13), 54.2 (*d*, C-9), 72.7 (*d*, C-12), 78.9 (*d*, C-3), 113.9 (*t*, C-16), 124.6 (*d*, C-14), 138.0 (*s*, C-8), 146.1 (*d*, C-15).

Yucalexin **B-22** [*ent*-2α,3β-dihydroxybeyer-15-en-12-one, 3.8 mg, Fr. **4**]. IR ν_{max}cm^{-1}: 3430 (OH), 1705 (C=O). EIMS 70 eV, *m/z* (rel. int.): 318 [M]$^+$ (66). 300 (19), 245 (20), 227 (23), 133 (26), 107 (100), 93 (43), 81 (34), 41 (38). ^{1}H NMR: see Table 1. ^{13}C NMR: δ 15.2 (*q*, C-20), 16.7 (*q*, C-19), 17.2 (*q*, C-17), 19.8 (*t*, C-6), 28.6 (*q*, C-18), 36.1 (*t*, C-7), 36.2 (*t*, C-11), 39.2 (*s*, C-4), 44.2 (*t*, C-1), 49.2 (*s*, C-8), 54.6 (*s*, C-10), 54.6 (*d*, C-5), 54.8 (*d*, C-9), 57.3 (*s*, C-13), 58.3 (*t*, C-14), 68.5 (*d*, C-2), 83.6 (*d*, C-3), 136.4 (*d*, C-16), 139.3 (*d*, C-15), 211.8 (*s*, C-12).

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Table 3. ^{1}H NMR data of *ent*-antisane family

Name	Element		H-1	H-2	H-3	H-5	H-18	H-19	H-20
A-16	**I**	ax	1.40 *ddd*	2.54 *ddd*		—		1.02 *s*	0.78 *s*
		eq	1.72 *ddd*	2.28 *ddd*			1.10 *s*		
			J 1a, 1e = 13.5, 1a, 2a = 13, 1a, 2e = 5.5, 1e, 2a = 6.5, 1a, 2e = 3, 2a, 2e = 16 Hz						
			0.78/1.40 (*W*-letter)						
A-19	**H**	ax	2.13 *dd*		3.90 *dd*	2.27 *dd*		0.68 *s*	0.57 *s*
		eq	2.28 *d*		3.40 *d*(OH)		1.20 *s*		
			J 1a, 1e = 12, 3a, 3OH = 5 Hz; 1a, 3a = 2 Hz (1, 3-diaxial)						
			0.57/2.13 (*W*-letter)						

Name	Element		H-9	H-11	H-12	H-13	H-14	H-15	H-17
A-16	**G**	ax	1.44 *dd*	2.18 *ddd*	2.32 *ddd*	6.14 *dd*	5.88 *d*	1.20 *d*	1.15 *s*
		eq		1.04 *ddd*				1.27 *d*	
			J 9, 11a = 9.5, 9, 11e = 6, 11a, 11e = 13, 11a, 12 = 3, 11e, 12 = 3 Hz;						
			12, 13 = 7, 13, 14 = 8 Hz (vicinal); 15a, 15e = 13 Hz						
A-19	**G**	ax	1.71 *dd*	2.16 *ddd*	2.32 *ddd*	6.12 *dd*	5.80 *d*	1.25 *d*	1.15 *s*
		eq		0.98 *ddd*				1.27 *d*	
			J 9, 11a = 9, 9, 11e = 6, 11a, 11e = 13, 11a, 12 = 3, 11e, 12 = 3 Hz;						
			12, 13 = 6.5, 13, 14 = 8 Hz (vicinal); 15a, 15e = 13 Hz						

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