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TRITERPENES FROM *Maytenus gonoclada* AND THEIR ATTRACTIVE EFFECTS ON *Tenebrio molitor*

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Bioactive metabolites have been isolated from plants of the genus *Maytenus* (Celastraceae), such as maytansinoids with insecticide activity, sesquiterpene pyridine alkaloids with insect antifeedant activity, and nortriterpene quinonemethides, which show antimicrobial activity [1]. Despite the great diversity of plant metabolites showing several biological activities, no compound with the friedelane skeleton presenting attractive-repellent effects has been described until now [2]. Previous phytochemical studies with aerial parts of Brazilian *Maytenus* species showed mainly pentacyclic triterpenes (PCTT) of the friedelane series, which are regarded as the characteristic constituents of plants belonging to this genus. It is worth noting that the PCTT were identified as the principal constituents of some Brazilian *Maytenus* sp. *Maytenus gonoclada* Martius (Celastraceae), which can be found in the "cerrado" region and rupestrian fields of Southeast and Northeastern Brazil. It is a polymorphic tree, usually spiny, the spines sometimes end in short shoots. The leaves alternate and are extipulate. The flowers are hermaphrodite in axillary, solitary, or fascicled dichotomous cymes; the calix is 4-5-lobed. The petals are 4–5, spreading. The stamens are 4–5, inserted on the margin of the disc or slightly below it; the filaments are slender and broad [3]. In this work we report the isolation, from the aerial parts of *M. gonoclada*, of seven compounds of the friedelane series: 3-oxofriedelane (**1**) [4], 3β-hydroxyfriedelane (**2**) [5], 3,11-dioxofriedelane (**3**), 3,16-dioxofriedelane (**4**) [6], 3-oxo-12α-hydroxyfriedelane (**5**) [7], 3-oxo-28-hydroxyfriedelane (**6**) [8], and 3-oxo-29-hydroxyfriedelane (**7**) [9]; two members of the lupane series, 3β-stearyloxy-lup-20(29)-ene (**8**) and lupeol (**9**) [10]; a taraxane, 3β-taraxerol (**10**) [11]; two ursanes, α-amyrin (**11**) [6] and 3β-stearyloxy-urs-12-ene (**12**) [12]; and oleanane β-amyrin (**13**) [13]. β-Sitosterol (**14**) [14], the polyol dulcitol or galactitol (**15**) [15], and palmitic acid (**16**) [16] were also chemically characterized. Two long-chain hydrocarbons with 28 and 36 carbons, respectively (**17**), were identified through high-resolution gas chromatography (GC). The hexane extract and constituents of *M. gonoclada* were tested for their attractive-repellent effects on the larvae of *Tenebrio molitor* (L.) (*Coleoptera*: Tenebrionidae). The hexane extract, the mixture of 3,11-dioxofriedelane and 3,16-dioxofriedelane, and the terpene 3-oxo-12α-hydroxyfriedelane showed attractive effects on these larvae.

Phytochemical studies demonstrated that the aerial parts of Brazilian *Maytenus* species consist mainly of triterpenes of the friedelane series, which are regarded as the characteristic constituents of plants belonging to this genus [2, 17]. It is worth noting that the pentacyclic triterpenes were identified as the principal constituents of Brazilian *Maytenus* sp. In the present work, we report the isolation of 13 known pentacyclic triterpenes: seven friedelanes (**1–7**), two lupanes (**8** and **9**), one taraxane (**10**), two ursanes (**11** and **12**), and the oleanane (**13**) obtained from *M. gonoclada*. Compounds **1** to **13** give a Liebermann–Buchard (LB) positive test for pentacyclic triterpenes, and **14** for steroids [18]. The ^{1}H NMR spectra of constituents **1–7** showed a hydrogen signal at ~ δ_H 0.87 (d, J = 6.8 Hz), which is in accord with the C-23 methyl group of members of the friedelane series. The ^{13}C NMR data confirmed that compounds **1–7** are friedelane derivatives [6]. The ^{1}H NMR spectra of compounds **8** and **9** showed signals at δ_H 4.6 and δ_H 4.7 that are characteristics of lupane triterpenes [19]. The presence of signals at δ_C 150.98 and δ_C 109.36 observed in ^{13}C NMR spectra confirmed the lupane skeleton of these compounds [10].

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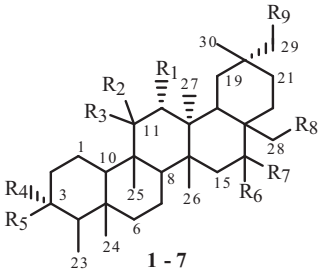

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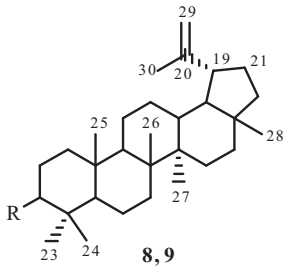

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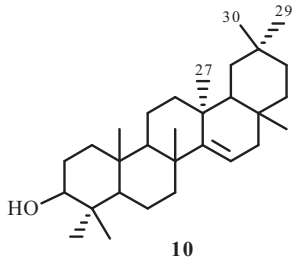

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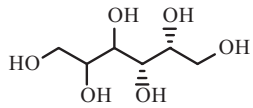

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1: $R_1 = R_2 = R_3 = R_6 = R_7 = R_8 = R_9 = H$, $R_4R_5 = O$; **2:** $R_1 = R_2 = R_3 = R_4 = R_6 = R_7 = R_8 = R_9 = H$, $R_5 = OH$
3: $R_1 = R_6 = R_7 = R_8 = R_9 = H$, $R_2R_3 = R_4R_5 = O$; **4:** $R_1 = R_2 = R_3 = R_8 = R_9 = H$, $R_4R_5 = R_6R_7 = O$
5: $R_1 = OH$, $R_2 = R_3 = R_6 = R_7 = R_8 = R_9 = H$, $R_4R_5 = O$; **6:** $R_1 = R_2 = R_3 = R_6 = R_7 = R_9 = H$, $R_4R_5 = O$, $R_8 = OH$
7: $R_1 = R_2 = R_3 = R_6 = R_7 = R_8 = H$, $R_4R_5 = O$, $R_9 = OH$; **8:** $R = H_3C(CH_2)_{15}CH_2COO$; **9:** $R = OH$

A double duplet at δ_H 2.30 and an intense signal at δ_H 1.25 suggested the presence of a side chain containing a carbonyl group in compound **8**. The double duplet at δ_H 5.46 (J = 8.5; 3.5 Hz), the signal of the quaternary carbon at δ_C 158.33, and the signal of the CH group at δ_C 117.11 allowed the identification of compound **10** as a taraxane triterpene [11, 20]. In the ^1H and ^{13}C NMR spectra of compound **11** were observed signals at δ_H 5.14 (J = 4 Hz) and at δ_C 79.23 characteristic of the ursane skeleton [6]. The signals of a multiplet at δ_H 5.3 associated with the olefin hydrogen and at δ_C 80.63 and at δ_C 173.71 observed in the NMR spectra indicate that compound **12** is a triterpene ester. The presence of signals at δ_C 124.36 and at δ_C 139.50 contributed to the identification of this ester as a derivative of α-amyrin, an ursane triterpene [12]. The signal at δ_H 5.19 (J = 4.0 Hz) characteristic of an olefin hydrogen and the signal of the hydroxylated carbon (C-3) at δ_C 79.02 confirmed the structure of the oleanane triterpene for **13**. In the ^1H NMR spectrum of compound **14** were observed a signal at δ_H 5.3 attributed to an olefin hydrogen and a multiplet at δ_H 3.5 corresponding to hydroxylated carbon. The profile of this spectrum was similar to those observed for the steroidal skeletons [21]. The chemical shift assignments of 28 signals observed in the ^{13}C NMR spectra, mainly the signals at δ_C 121.74 and δ_C 140.79 attributed to olefin carbons, contributed to confirming the structure of **14**. The IR spectra of compound **15** indicate the presence of the hydroxyl group through the absorption band at 3450–3253 cm^{-1}. The aliphatic character of this substance was confirmed by the absorptions at 2840–2800 cm^{-1} (ν C-H) and 1450–1370 cm^{-1} (δ C-H). There were also observed intense absorptions bands at 1048 cm^{-1} characteristic of CH_2-O of hydroxyl bonded to primary carbon, and at 1100 cm^{-1} due to deformation of the R_2CH-O bond of hydroxyl attached to the secondary carbon. The IR data together with melting point and literature data confirmed the structure of compound **15**. The IR spectra of compound **16** showed an absorption band at 1698 cm^{-1} characteristic of the stretch of the CO double bond of the carboxylic acid involved in hydrogen bonding and at 1348–1187 cm^{-1} characteristic of the angular deformation in the plane of the CH_2 groups, commonly found in long-chain fatty acids [22]. In the ^1H NMR spectrum of compound **16** were observed a triplet at δ_H 2.34 (J = 7.6 Hz) assigned to the hydrogen adjacent to the carbonyl and the signal at δ_H 0.88 (t, J = 6.6 Hz) associated with the methyl hydrogens. In the ^{13}C NMR spectrum of 16 the signal at δ_C 179.51 was attributed to the carbonyl of carboxylic acid. In the GC-MS analysis we detected a molecular ion peak (*m/z* 256.15) consistent with the mass of palmitic acid. The IR spectrum of compound **17** showed intense absorption bands in the region of 2950 to 2800 cm^{-1} and 1470 to 1458 cm^{-1}, and a double band at 730–720 cm^{-1}. The observed profile of this spectrum suggested the aliphatic nature of substance (**17**). By analysis the chromatogram obtained by GC for compound **17** and comparison with authentic standards of hydrocarbons, we were able to identify the presence of two long-chain hydrocarbons with C-28 and C-36 in sample **17**. The constituents isolated (**1** to **17**) from leaves and branches of *M. gonoclada* were identified by melting point, comparison of the GC data with authentic samples, and through NMR spectrometry, in which the experimental spectral results were compared with the literature data. Members of the friedelane, oleanane, and ursane series are constituents most frequently isolated from species of the Celastraceae family [12]. Thus, the structures of the constituents found in the present study of *Maytenus gonoclada* resemble those that were previously isolated from other species of *Maytenus*, such as *M. imbricata* [2] and *M. acanthophylla* [15]. The isolation of known metabolites in the species studied here for the first time contributes to the development of biosynthetic routes and the establishment of relationships between the species, thus contributing to chemotaxonomy of the species. Three substances isolated from *M. gonoclada* showed attractive effects on *T. molitor* larvae (Table 1).

TABLE 1. Attractive Effect of *Tenebrio molitor* Larvae Induced by Samples, Observed after Three Hours of Exposure Time

Dose, % w/v	Time of exposure, χ^2-test					
	1 h	2 h	3 h	1 h	2 h	3 h
	Hexane extract			3-Oxofriedelane		
1.453	10.4	10.4	12.0	2.4	2.4	7.2
0.726	7.2	10.4	7.2	2.4	10.4	7.2
0.363	8.8	13.6	16.8*	4.0	7.2	7.2
0.182	8.8	10.4	13.6	5.6	4.0	7.2
0.091	5.6	8.8	12.0	4.0	5.6	10.4
0.045	7.2	12.0	10.4	0.8	7.2	2.4
0.023	7.2	0.8	2.4	12.0	2.4	5.6
Dose, % w/v	Mixture of 3,11-dioxofriedelane and 3,16-dioxofriedelane			3-Oxo-12α-hydroxyfriedelane		
1.453	13.6	16.8*	20.0*	5.6	8.8	13.6
0.726	16.8*	12.0	8.8	7.2	16.8*	8.8
0.363	4.0	8.8	4.0	12.0	10.4	12.0
0.182	12.0	5.6	8.8	10.4	12.0	10.4
0.091	12.0	10.4	8.8	8.8	12.0	7.2
0.045	13.6	13.6	8.8	7.2	7.2	12.0
0.023	8.8	7.2	13.6	0.8	0.8	10.4

*Data tested by applying the χ^2-test. χ^2-tabulated is 14.2. Significant result was considered when χ^2-test > χ^2-tabulated.

In the experimental conditions we did not observed significant attractive effects from 3-oxofriedelane, but the hexane extract showed significant activity at a dose level of 0.363% (w/v) after 3 h of exposure. The mixture of 3,11-dioxofriedelane and 3,16-dioxofriedelane at the 0.726% (w/v) dose level showed significant attractive activity after 1 h, and 3-oxo-12α-hydroxyfriedelane [at the 0.726% (w/v) dose level] after 2 h of exposure. Considering that these extracts and constituents showed attractive effects, they can be used in traps for the control of *T. molitor* infection of stored supplies. No doubt, extracts of plants and secondary metabolites are a very interesting alternative in pest control; in addition, only a small number of plants have been evaluated in relation to the natural source available worldwide, so there are important incentives for future research.

Plant Material. The aerial parts of *M. gonoclada* were collected in "Serra da Piedade" Mountain at Caete, Minas Gerais, Brazil in October 2004. The plant material was identified by Dra. Rita Maria Carvalho-Okano of Universidade Federal de Vicosa, MG, Brazil. A voucher specimen (No. HBCB 60280) is preserved in the Herbarium of Instituto de Ciencias Biologicas, ICB, UFMG (Belo Horizonte, Minas Gerais, Brazil).

Evaluation of the Attractive-Repellent Effects. The extracts and constituents of *M. gonoclada* were dissolved in acetone with DMSO-d_6 (0.5%), and the final test solutions were prepared in concentrations of 1.453, 0.726, 0.363, 0.182, 0.091, 0.045, and 0.023% w/v. On Petri dishes (20 cm diameter), filter paper disks (2 cm diameter) were placed in opposing positions, and over it, sterilized corn bran (0.1 g). In one of the filter paper disk was applied 5.0 µL of sample solution. The solvent was allowed to evaporate before testing. Five larvae (2 day old) were put on each Petri dish, and the assay was carried out under laboratory conditions of temperature (25 ± 5°C) and relative humidity (65 ± 5%). Four replicates were run for each tested concentration, so that 20 larvae/concentration were assayed.

Insect and Culture Rearing Conditions. *T. molitor* L. used in the bioassay were obtained from stock culture that had been maintained on wheat bran, corn, and dried bread (1:1:1) in wood boxes (70 cm × 40 cm × 20 cm) in darkness and under controlled conditions of temperature (25 ± 5°C) and relative humidity (65 ± 5%). Periodically, larvae and adults were separated from the substrate to keep the colony clean.

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