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GC/MS investigations of the minor constituents of *Piper guineense* stem

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Chemical investigations by GC/MS-analysis of stem extracts of *Piper guineense* resulted in the detection and identification of thirty-nine new constituents of the stem, apart from previously isolated constituents. These are isobutyl, pyrrolidyl and piperidyl amide alkaloids. Fifteen new natural products have been identified. Four of these natural products have been designated *iyeremide* A and B (these are pyrrolidine and piperidine analogues of *pellitorine*) and *cycloguineense* A and B, which are also piperidine analogues of cyclostachine A and B. There is a need to confirm the structures of some of these new constituents by synthesis. Apart from these amide alkaloids, many volatile oil components-monoterpenes, sesquiterpenes, terpenoids, lignans and sterols – were detected.

1. Introduction

Piper guineense Schum and Thonn, Piperaceae, is a rain forest woody climber found in the southern parts of Nigeria where it usually grows as a tree-top canopy. It is known as "iyere" in south-western Nigeria. The plant has also been reported [1] in other parts of West and central Africa particularly in Ghana and Cameroon. The plant has a reputation for its medicinal values – the leaves, fruits and roots are ingredients in herbal drug preparations for coughs, colds, bronchitis, venereal diseases, intestinal disorders, tooth-ache, rheumatism, skin problems and insect infestation. The leaves and fruits are eaten and have been used as condiments, flavorants and generally as spices in foods. The sharp peppery taste of the fruit has contributed to its acceptability and use in some food and drug preparations.

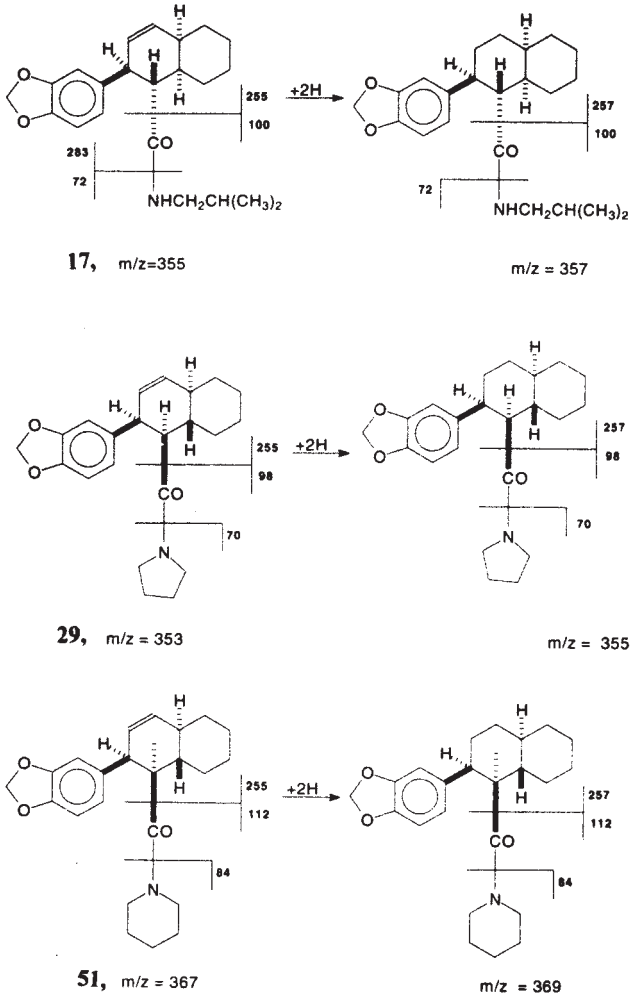
Previous chemical studies of the plant whole stem and roots have resulted in the isolation and identification of novel amide alkaloids [2–5]. This present work was undertaken to examine the chemical constituents of the stem, a part that has not been examined thoroughly before.

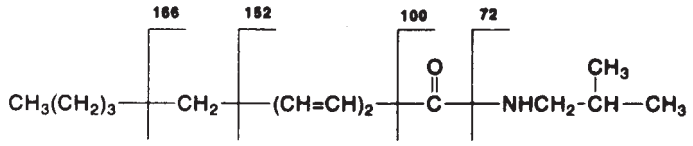
2. Investigations, results and discussion

On GC/MS examination the pulverized stem pentane extract revealed the presence of some monoterpene hydrocarbons (characterized by 9 or more peaks at mass units 134, 136, identified as pinene, *p*-cymene, limonene, phellandrene etc.), sesquiterpene hydrocarbons and alcohols or ketones (characterized by 36 or more peaks at mass units 204, 220, 222, identification-MS library-elemene, copaene, guaiene, caryophyllene, cadinene and their derivatives etc.) and free fatty acids which were eluted from the column within the first 45 min of the programme. Eluted along with these volatile oil constituents were benzoic acid ($GC_{tR} = 17.66$ min, M^+ 122), thymol ($GC_{tR} = 27.90$ min, M^+ 150), piperonal ($GC_{tR} = 25.06$, M^+ 150), vanillin ($GC_{tR} = 27.90$ min, M^+ 152), 3,4-methylenedioxybenzoic acid ($GC_{TR} = 32.69$ min, M^+ 166) and the more volatile amide alkaloids.

A list of 51 amide alkaloids identified includes 18 isobutylamides, 14 pyrrolidides and 19 piperidides. The identification was made easier because these amides are well-suited to GC-MS analysis – the amides are well separated on the 30 m long column and MS provides enough structural information to permit the deduction of structures of interest.

For all the compounds, both aliphatic and aromatic, prominent and significant peaks in the MS could be accounted for with fragmentation at the N–CO bond followed by cyclisation, fissions and hydrogen transfers as noted earlier [4, 7, 8]. All compounds gave parent ions. This is particularly important for the *Piper trichostachyon* alkaloids **17**, **29**, and **31**. The MS fragmentation observed for cyclopiperstachine (**17**), cyclostachine (**29**) and the new natural product, cycloguineense **B** (**51**) and their hydrogenated products can be rationalized, following the scheme of Joshi et al. [9]. The major ions for **51** and its enantiomer, cycloguineense **A** (**50**) occurred at m/z 255,





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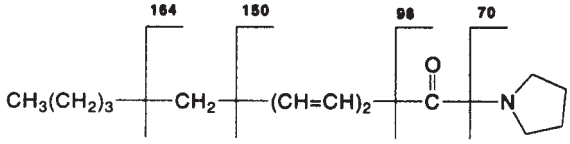

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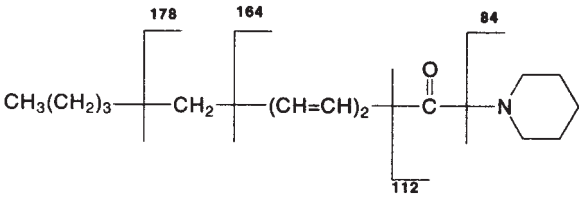

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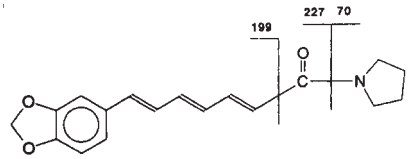
232, 135 and 112. Piperine (**46**), wisanine (**48**), piperlonguminine (**16**), trichostachine (**25**), the isobutylamides and their derivatives are well-known and their fragmentation patterns are well understood. Compounds with pyrrolidyl and piperidyl moieties in their molecules also offer neat fragmentation patterns for the recognition of the moieties in their spectra.

This work represents a first report of the occurrence of pyrrolidine and piperidine analogues of pellitorine in nature for which we are proposing the trivial names *iyeremide* A for *N*-pyrrolidyl-2,4-decadienamide (**20**) and *iyeremide* B for *N*-piperidyl-2,4-decadienamide (**36**) because of their structural relationship to pellitorine (**3**) [11], the well-known pungent and insecticidal component of *Anacyclus pyrethrum*. Of special interest also are the small molecules **19** and **33** which are also new natural products and, whose structures we have confirmed by synthesis. *Piper guineense* has also furnished 1-piperettyl pyrrolidine (**32**) the higher homologue of trichostachine (**25**) and piperettine (**49**), the higher homologue of piperine (**46**) earlier isolated from *Piper nigrum* [12].

19 **33**



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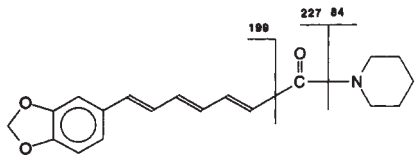

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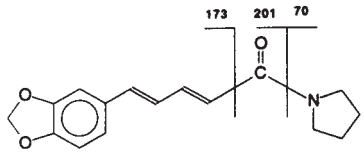

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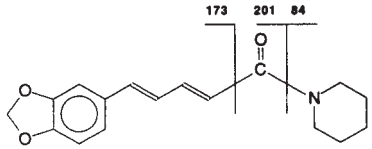

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3. Experimental

3.1. General procedures

Melting points were uncorrected. NMR (^{1}H, ^{13}C) experiments were recorded in $CDCl_3$ on a Gemini 200 MHz using TMS as an internal standard and chemical shifts are shown as δ-values (ppm). MS (as direct inlet, EI at 70 eV, ion source at 200 °C) were recorded as m/z (rel. int) on a FINNIGAN MAT instrument, Model GCQ-Mass spectrometer, serial No GQ 200037 fitted with Model GCQ-Gas chromatography and on a J & W Scientific high resolution GC column, DB-5, 30 metres long with I.D (mm), 0.25, film (μm) 0.25, serial No US 1402756H. Carrier gas He: 10 psi-20 psi with conditions: T_1, 50 °C (2 min), programme 3 °C/min until T_2, 270 °C (30 min). TLC was carried out using silica gel 60 F_{254} (Merck) and solvents for TLC were I, Tol./EtOAc (4 + 1), II, Tol./EtOAc (1 + 1) and (111), $CHCl_3$/MeOH (9 + 1); detection: UV-light. IR spectra were recorded as KBR pellets.

3.2. Plant material

The *Piper guineense* stem referred to in this work was the climbing part of the plant when it climbed a tree to form a canopy or the sprawling portion when it could not get a tree to climb. The stem was sourced routinely from a plant growing in Igbaye (Osun State, Nigeria) and was taxonomically identified by Dr. H. C. Illoh (Dept. of Botany, Obafemi Awolowo University) who also kept a herbarium specimen, IFE Herb 264. The first collection was made in October, 1995. Freshly-collected plant material was dried in an aerated oven at 45 °C before communition to powder for chemical processing.

3.3. Extraction, isolation and identification procedures

For GC/MS investigations, pulverized whole stem (210 g) was covered with methanol (A.R. grade, 1 l.) with occasional shaking and extracted for 72 h. at RT. MeOH was removed under reduced pressure (bath temp. 40 °C) to leave a residue (4.61 gm). This residue was triturated in MeOH/H_2O (1 : 1) and filtered. The mixture was extracted with pentane and afterwards with $CHCl_3$. Each extract was washed with NaCl, dried over anh. Na_2SO_4, filtered and reduced to low volume. Samples of the residue from the pentane extract (630 mg) and $CHCl_3$ (1.04 g) were separately subjected to GC-MS analysis.

To isolate some constituents, pulverized whole stem (1.84 kg) was exhaustively extracted in MeOH for 96 h in the dark. MeOH was removed at reduced pressure (water bath temp. at 40 °C) to leave a residue (183.93 g). This residue was taken up in a MeOH/H_2O (1 : 1) mixture, filtered and extracted with $CHCl_3$ (500 ml × 3). The combined $CHCl_3$ extract was washed with NaCl, dried over anh. Na_2SO_4, filtered and concentrated to dryness, to leave a residue (46.80 g). Portions of this residue were chromatographed over silica gel (pTLC, S_1O_2 60F_{254}, IMM, Solv 1.). A total of six fractions designated PgstO1-6 were obtained. The corresponding fractions were separately eluted in $CHCl_3$ and monitored by TLC and GC-MS. The concentration of some fractions resulted in some deposits of constituents which were purified further by crystallisation while others were re-chromatographed in solv. II. for further purification and isolation of constituents.

Alkaline hydrolysis: Compound in 10% alcoholic KOH was heated under reflux for 48–50 h and crystals in the form of K salt were collected by filtration, dissolved in minimum amount of H_2O and acidified with dil. HCl. Precipitates were usually extracted in $CHCl_3$ for further analysis. The alcoholic filtrates were usually acidified and evaporated to dryness before further purification and analysis.
Catalytic hydrogenation: Pd–C catalyst (20 mg) was usually added to the sample or mixture (200 mg) in dry MeOH (10 ml) at normal atmospheric conditions with the mixture stirred under N_2 and then H_2 until the uptake of H_2 was complete. The reaction mixture was filtered and evaporated *in vacuo* to yield the hydrogenated product which was also analysed by GC-MS and TLC.
Synthesis: Extensive syntheses were carried out following the procedure used earlier [6].
In this work and for most constituents of low to medium concentrations, structural determinations were based on their MS, hydrogenation products, chromatographic behaviour and comparison with synthetic analogues. For those at fairly high concentrations, we have relied, in addition to the above, on m.pt determinations, hydrolysis products and spectroscopic data. Retention times of synthetic compounds, compounds previously isolated by us and some locally-purchased products were compared with those of our isolates to confirm the structures. In four cases, we found it convenient to mix an authentic sample with an isolate to prove identity.

3.4. Analysis of constituents

The whole stem pentane extract representing about 13.7% of the total MeOH extract contained the volatile oil components – the terpenes, terpenoids, fatty acids, benzoic acid derivatives and many of the amides. Fractions Pgst05 and 06 also contained these compounds.

3.4.1. N-Pyrrolidyl-1-acetylamide (**19**)

Detected in Pgst01. GC: $t_R = 16.28$ min-EIMS (m/z, % int.): 113 (M^+, 73), 98 (CO-Pyrrolidyl, 100), 70 (Pyrrolidyl, 87), 84 (40), 55 (75). This new natural product is identical with a synthetic specimen obtained from the reaction of acetylchloride and pyrrolidine.

3.4.2. N-Piperidyl-1-acetylamide (**33**)

Detected as a minor component in Pgst01. GC: $t_R = 19.04$ min – EIMS (m/z, % int.): 127 (M^+, 80), 112 (CO-Piperidyl, 43), 84 (Piperidyl, 100), 70 (72), 56 (66), 67 (20), 85 (15). This new compound is identical with a synthetic specimen obtained from the reaction of acetylchloride and piperidine.

3.4.3. N-Isobutyl-2,4-octadienamide (**1**)

Detected from fraction Pgst05. GC: $t_R = 40.63$ min – EIMS (m/z, % int.): 195 (M^+, 42), 180 (M^+-CH_3, 45), 152 (M^+-C_3H_7, 56), 123 (M^+-N-isobutyl, 98), 113 (90), 96 (M^+-CO–N-isobutyl, 100), 81 (65), 67 (60). Catalytic hydrogenation produced N-isobutylcapramide, EIMS (m/z, % int.): 199 (M^+) for $C_{12}H_{25}NO$.

3.4.4. N-Isobutyl-2-decenamide (**2**)

Detected in fraction Pgst05 as a new product. GC: $t_R = 45.43$ min – EIMS (m/z, % int.): 225 (M^+, 12), 210 (M^+-CH_3, 38), 182 (M^+-$CH(CH_3)_2$, 45), 153 (M^+-N-isobutyl, 100), 154 (26), 126 (M^+-$CH_3(CH_2)_6$-, 69), 140 (M^+-$CH_3(CH_2)_5$-, 15), 69 (70). The strong ions at m/z, 126 and 140 confirm the position of the double bond in this new compound. Catalytic hydrogenation gave a product GC: $t_R = 43.18$ min, MS: 227 (M^+, 5), 115 (71), 100 (55), 172 (30), 128 (28), 184 (19), 72 (24), 60 (100), 142 (10).

3.4.5. N-Isobutyl-2,4-decadienamide (pellitorine) (**3**)

Detected in fraction Pgst05. GC: $t_R = 48.15$ min – EIMS (m/z, % int.): 223 (M^+, 34), 208 (M^+-CH_3, 40), 180 (M^+-$CH(CH_3)_2$ 124), 151 (M^+-N-isobutyl, 70), 152 (M^+-$CH_3(CH_2)_4$, – 59), 96 (100), 113 (75), 95 (73). Catalytic hydrogenation gave a product as with **2**.

3.4.6. N-Piperidyl tetrahydrodecanamide (**34**)

Detected in fraction Pgst04. GC: $t_R = 49.69$ min – EIMS (m/z, % int.): 239 (M^+, 7), 127 (M^+-CO-Piperidyl, 100), 140 (M^+-$CH_3(CH_2)_5$-, 46), 112 (CO-Piperidyl, 60), 84 (Piperidyl), 70 (M^+-$(CH_2)_4$ CO-Piperidyl, 54), 154 (9), 99 (5), 69 (15). The compound behaved like a saturated aliphatic amide giving a base peak at m/z 127 arising from the fission of the bond beta to the carbonyl group, followed by McLafferty rearrangement.

3.4.7. N-Isobutyl-3,4-dimethoxybenzoic acid amide (**14**)

5 mg obtained from fraction Pgst04. GC: $t_R = 51.54$ min – EIMS (m/z), % int.): 237 (M^+, 6), 222 (M^+-CH_3, 50), 194 (10), 181 (55), 180 (40), 166 (62), 151 (45), 150 (25), 138 (40), 110 (100) Identical (IR, M.pt. TLC) with the synthetic product obtained from the reaction of isobutylamine and 3,4-dimethoxybenzoic acid.

3.4.8. N-Isobutyl-2,4-hendecadienamide (**4**)

Detected as a trace in Pgst05. GC: $t_R = 51.80$ min – EIMS (m/z, % int.): 237 (M^+, 21), 222 (M^+-CH_3, 23), 208 (M^+-CH_2CH_3, 25), 194 (M^+-$CH(CH)_2$, 13), 180 (M^+-$CH_2CH(CH_3)_2$, 32), 166 (M^+-N-isobutyl, 74), 151 (M^+-$CH_3(CH_2)_5$,95), 152 (25), 96 (100), 81 (70).

3.4.9. N-Piperidyl-2-decenamide **(35)**

Detected in Pgst02. GC: $t_R = 51.94$ min – EIMS (m/z, % int.): 237 (M^+, 5), 222 (M^+-CH_3, 2), 208 (M^+-C_2H_5, 7), 194 (M^+-$CH_3(CH_2)_2$, 8), 180 (M^+-$CH_3(CH_2)_5$-, 9), 84 (12), 138 (M^+-$CH_3(CH_2)_6$-, 100), 127 (M^+-CO-Piperidyl, 18). The strong ions at m/z, 138 and166 confirm the position of the double bond at C-2 for this new compound.
Catalytic hydrogenation gave a product identical with **34**.

3.4.10. N-Pyrrolidyl-1-cinnamoylamide (**23**)

11 mg obtained from fraction Pgst02. GC: $t_R = 52.69$ min – EIMS (m/z, % int.): 201 (M^+, 100), 131 (M^+-CO-Pyrrolidyl, 90), 103 (for PhCH=CH, 87), 77 (Ph, 44), 70 (Pyrrolidyl, 35), 91 (55), 172 (25), 200 (33), 115 (14). New constituent, identical (UV, IR, NMR) with a synthetic.

3.4.11. N-Isobutyl-3-(3,4-methylenedioxyphenyl)propionamide (= dihydrofagaramide) (**11**)

10 mg obtained from Pgst02. GC: $t_R = 53.04$ min – EMIS(m/z,% int.): 249 (M^+, 25), 192 (M^+-$CH_2CH(CH_3)_2$, 95), 177 (M^+-N-isobutyl, 100), 145 (67), 137 (17), 117 (20), 89 (30), 193 (14). This is a first report of its occurrence as a natural product, Strong ions at m/z 177, 192, 249 and 137 confirm its structure. Identical (NMR, MS, TLC) with a synthetic.

3.4.12. N-Pyrrolidyl-2,4-decadienamide (**20**) – Iyeremide A

Obtained from fraction Pgst02. GC: $t_R = 53.74$ min – EIMS (m/z, % int.): 221 (M^+, 15), 98 (CO-Pyrrolidyl, 17), 150 (M^+-$CH_3(CH_2)_4$-, 100), 164 (M^+-$CH_3(CH_2)_3$-15), 178 (M^+-$CH_3(CH_2)_2$-, 20), 192 (15), 206 (M^+-CH_3,5), 151 (20), 70 (22). It is a new natural product, the pyrrolidine analogue of pellitorine (**3**) and it is being designated iyeremide A. Catalytic hydrogenation gave a product GC$t_R = 48.49$ min – EIMS {m/z, % int.} 225 (M^+, 3), 113 (100), 70 (66), 98 (30), 126 (40) as expected.

3.4.13. N-Piperidyl-2,4-decadienamide (**36**) – Iyeremide B

Obtained from fraction Pgst02. GC: $t_R = 54.44$ min – EIMS (m/z, % int.): 235 (M^+, 12), 206 (M^+-CH_3CH_2, 15), 192 (M^+-$CH_3(CH_2)_2$-, 100) 178 (M^+-CH_3CH_2-, 40) 164 (M^+-$CH_3(CH_2)_4$-, 72), 150 (M^+-Piperidyl, 45), 138 (M^+-$CH_3(CH_2)_4(CH{=}CH)$-, 62), 84 (Piperidyl, 75). This new compound is the piperidyl analogue of pellitorine (**3**) and it is designated iyeremide B. After catalytic hydrogenation identical with **34**.

3.4.14. N-Isobutyl-2,4-dodecadienamide (**5**)

Detected in Pgst04. GC: $t_R = 54.65$ min – EIMS (m/z, % int.): 251 (M^+, 10), 236 (M^+-CH_3, 12), 179 (M^+-N-Isobutyl, 42), 152 (M^+ – $CH_3(CH_2)_6$–, 40), 113 (45), 96 (100), 81 (52), 166 (15). Catalytic hydrogenation gave a product with GC$t_R = 49.96$ min – EIMS {m/s, % int.}: 255 (M^+, 4), 60 (100), 115 (80), 100 (48), 128 (27), 200 (24),(17), 212 (10), 170 (8), 142 (8)

3.4.15. N-Isobutyl-2,4-tridecadienamide (**6**)

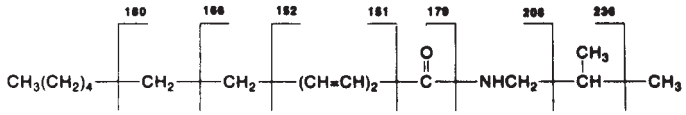
Obtained from Pgst05. GC: $t_R = 59.72$ min – EIMS (m/z, % int.): 265 (M^+, 14), 250 (M^+-CH_3, 16), 222 (M^+-$CH(CH_3)_2$, 15) 236 (M^+-CH_3–CH_2, 22) 209 (M^+-$CH_3CH(CH_3)_2 + H$, 18), 179 (M^+-CH_2-N-Isobutyl, 100), 166 (for $CH_3(CH_2)_7(CH{=}CH)_2$-, 80), 152 (M^+-$CH_3(CH_2)_7$-, 25), 138 (16) 127 (43), 110 (52). Catalytic hydrogenation gave a product, EIMS (m/z, % int.): 269 (M^+, 3), 268 (M^+-1,6), 256 (7), 115 (100), 60 (83), 100 (50), 128 (30), 72 (20), 142 (10), as expected.

3.4.16. N-Isobutyl-3,4-methylenedioxycinnamoylamide (= fagaramide) (**13**)

13 mg isolated from Pgst04. GC: $t_R = 60.04$ min – EIMS (m/z,% int.): 247 (M^+, 26), 190[M^+-$CH_2CH(CH_3)_2$, 100], 175 (M^+-N-isobutyl, 92), 145 (81), 117 (30), 89 (61), 63 (16), 232 (2). Previously isolated by us [20], identical (UV, IR, NMR) with an authentic sample. New constituent of the stem.

3.4.17. N-Pyrrolidyl-2,4-dodecadienamide **(21)**

Identified in Pgst02. GC: $t_R = 60.09$ min – EIMS (m/z, % int.): 249 (M^+, 8), 98[CO-Pyrrolidyl, 23), 150 (M^+-$CH_3(CH_2)_6$ – 100), 164 (M^+-$CH_3(CH_2)_5$-, 14), 178 (M^+-$CH_3(CH_2)_4$-, 17), 124 (6), 179 (M^+-Pyrrolidyl, 6), 113 (28), 70 (Pyrrolidyl, 20).



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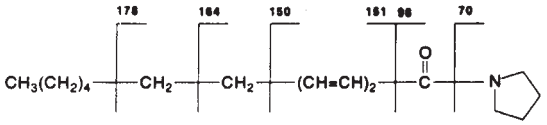

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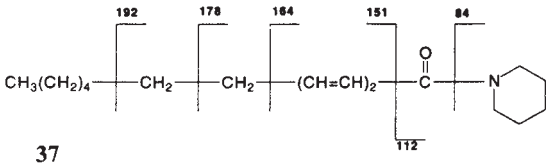

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3.4.18. N-Pyrrolidyl-3-methoxycinnamoylamide (**24**)

4 mg isolated from Pgst02. GC: $t_R = 60.38$ min – EIMS (m/z, % int.): 231 (M^+, 25), 161 (M^+-Pyrrolidyl, 100), 162 (41), 163 (4), 133 (M^+-CO-Pyrrolidyl, 43), 131 (70), 118 (38), 103 ($PhOCH_3$, 28), 90 (30), 89 (21), 77 (15), 70 (80). Known natural product, identical with synthetic product and published data [13]. A new constituent in this plant.

3.4.19. N-Isobutyl-2,4-tetradecadienamide (**7**)

Identified in Pgst05. GC: $t_R = 60.63$ min – EIMS (m/z,% int.): 279 (M^+, 42), 264 (M^+-CH_3, 38), 236 (M^+-$CH(CH_3)_2$, 18), 207 (M^+-N-isobutyl, 62), 179 (8), 152 (M^+-$CH_3(CH_2)_8$-, 62), 96 (100), 113 (50), 166 (21), 180 (20). First report in this plant.

3.4.20. N-Piperidyl-2,4-dodecadienamide (**37**)

Identified as a minor component of the pentane fraction. GC: $t_R = 60.67$ min – EIMS (m/z,% int.): 263 (M^+, 10), 234 (M^+-CH_2CH_3, 8), 220 (M^+-$CH_3(CH_2)_2$-, 10), 192 (M^+-$CH_3(CH_2)_4$, 100), 178 (M^+-$CH_3(CH_2)$ – 41), 164 (for $(CH{=}CH)_2CO$- Piperidyl, 82), 151 (M^+-CO-Piperidyl, 12), 150 (38), 112 (15), 84 (86), 79 (53).

3.4.21. N-Isobutyl-5-(3,4-methylenedioxyphenyl)-2-pentenamide (= Δαβ-dihydropiperlonguminine) (**12**)

7 mg isolated from Pgst02. Identified as a minor component of the pentane fraction.
GC: $t_R = 61.82$ min – EIMS (m/z, % int.): 275 (M^+, 5), 218 (M^+-$CH_2CH(CH_3)_2$, 2), 203 (M^+-N-isobutyl, 2), 175 (M^+-CO−N-isobutyl, 16), 135 (M^+-$CH_2CH{=}CH{-}CO{-}$N-isobutyl, 100) 147 (2), 77 (20), 136 (10), 174 (17). Identical (UV, M.pt. IR, TLC) with an authentic sample. Known constituent [21] of the fruit. Hydrogenated product had $GC_{tR} = 60.69$ min – EIMS [(m/z, % int.): 277 (M^+, 30), 204 (86), 205 (27), 176 (45), 161 (20), 148 (100), 135 (70), 100 (20), 72 (18), 60 (45), 115 (25), 128 (18), confirming its structure.

3.4.22. N-Isobutyl-5-(3,4-methylenedioxyphenyl)-3-pentenamide (= Δβγ-dihydropiperlonguminine) (**15**)

Detected in Pgst02. GC: $t_R = 62.11$ min – EIMS (m/z, % int.): 275 (M^+, 20), 232 (M^+-$CH(CH_3)_2$, 2), 204 (M^+-N-isobutyl, 9), 176 (63), 175 (50), 140 (100), 161 (40, 135 (45), 131 (50), 117 (40), 116 (41), 115 (38), 103 (11). The strong ions at m/z 135 and 161 confirm the double bond in this isomer at C-3. First report as a natural product. Identical with the synthetic specimen published by Loder et al. [8].

3.4.23. N-Isobutyl-5-(3,4-Methylenedioxyphenyl)-2,4-pentadienamide (= piperlonguminine) (**16**)

It is a major component of Pgst03, 14 mg isolated. GC: $t_R = 64.25$ min – EIMS (m/z, % int.): 273 (M^+, 55), 230 (2), 217 (7), 216 (M^+-$CH_2CH(CH_3)_2$, 17), 201 (M^+-N-isobutyl, 56), 173 (M^+-CO−N-isobutyl, 78), 174 (40), 172 (30), 143 (45), 135 (44), 115 (100), 89 (12), 77 (12), 96 (10). Identified [16] previously from the fruit, after catalytic hydrogenation identical with the product from (**12**), known component [14] of *Zanthoxylum lemairie.*

3.4.24. N-Pyrrolidyl pentadecanoylamide (**22**)

GC: $t_R = 64.71$ min – EIMS (m/z, % int.): 296 ($M^+ + 1$, 40), 225 (M^+ – 2H-Pyrrolidyl, 70), 167 (for $CH_3(CH_2)_{11}$-, 25), 139 (for $CH_3(CH_2)_9$-,20), 195 (M^+-CO-Pyrrolidyl, 100), 70 (Pyrrolidyl, 12).

3.4.25. N-Pyrrolidyl-5-methoxy-3,4-methylenedioxycinnamoylamide (**28**)

Detected in the pentane extract. GC: $t_R = 65.27$ min – EIMS (m/z, % int.): 275 (M^+, 24), 276 (7), 205 (M^+-70, 4), 204 (30), 177 (M^+-CO-70, 5), 176 (32), 175 (14), 151 (3), 148 (58), 140 (14), 135 (60), 126 (44), 113 (100), 103 (17), 98 (37), 91 (26), 77 (50), 70 (57). After hydrogenation, gave a product at m/z 277 (M^+, 21), 165 (100), 166 (19).

3.4.26. N-Isobutyl-2,4-hexadecadienamide (**8**)

7 mg isolated from Pgst02. GC: $t_R = 66.36$ min – EIMS (m/z, % int.): 307 (M^+, 40), 292 (M^+-CH_3, 42), 264 (M^+-$CH(CH_3)_2$, 10), 235 (for $CH_3(CH_2)_{10}(CH{=}CH)_2CO$-, 70), 152 ($M^+$-$CH_3(CH_2)_{10}$, 100), 98 (96), 113 (82), 96 (80), 81 (67), 126 (35), 166 (25), 180 (22), 236 (20).
Isolated previously from the fruit. Catalytic hydrogenation gave a product, GC: $t_R = 61.99$ min – EIMS (m/z, % int.): 311 (M^+, 8), 268 (6), 256 (11), 239 (3), 226 (3), 184 (14), 170 (10), 142 (10), 128 (30), 115 (100), 100 (50), 72 (20), 60 (83).

3.4.27. N-Piperidyl pentadecanoylamide (**39**)

Detected in the pentane extract. GC: $t_R = 66.40$ min – EIMS (m/z, % int.): 308 (M^+-1, 30), 309 (M^+, 25), 226 (M^+-Piperidyl, 22), 224 (20), 195 (M^+-CO-Piperidyl, 53), 167{$CH_3(CH_2)_{11}$-, 53}, 139{$CH_3(CH_2)_9$-, 55}, 84 (Piperidyl, 100).

3.4.28. N-Piperidyl-5 (3,4-methylenedioxyphenyl)-pentanolamide (=tetrahydropiperine) (**45**)

Detected in Pgst04. GC: $t_R = 66.50$ min – EIMS (m/z, % int.): 289 (M^+, 63), 204 (93), 205 (5), 176 (26), 177 (4), 148 (65), 154 (40), 140 (40), 137 (100), 135 (32), 112 (76), 103 (16, 86 (53), 84 (52), 70 (91), 67 (37). First report as a natural product. Data identical with that published [8] for a synthetic specimen.

3.4.29. N-Piperidyl-2,4-tetradecadienamide (**38**)

Detected in Pgst04. GC: $t_R = 66.57$ min – EIMS (m/z, % int.): 291 (M^+, 8), 234 (M^+-$CH_3(CH_2)_3$-, 5), 206 (M^+-$CH_3(CH_2)_5$-, 24), 274 (M^+-CH_3, 10), 192 (M^+-$CH_3(CH_2)_6$-, 85), 178 ($CH_2(CH{=}CH)_2{-}CO$-Piperidyl, 28), 164 (M^+-$CH_3(CH_2)_8$- 70), 138 (CH=CH−CO-Pipridyl, 100), 112 (CO-Piperidyl, 32), 84 (Piperidyl, 90). Hydrogenated product had GC: $t_R = 62.19$ and major ions at m/z 295 (M^+, 3), 127 (100), 112 (60), 140 (33), 84 (35), 70 (37), 86 (18), 182 (6).

3.4.30. N-Pyrrolidyl-dimethoxycinnamoylamide (**27**)

5 mg isolated from fraction Pgst04. GC: $t_R = 67.21$ min – EIMS (m/z, % int.): 261 (M^+, 10), 191 (M^+-70, 55), 192 (100), 164 (M^+-CO-Pyrrolidyl, 67), 163 (28), 148 (35), 70 (37). Identical (UV, IR, NMR) with a synthetic specimen.

3.4.31. N-Piperidyl-5-(3,4-methylenedioxyphenyl)-2-pentenamide (= Δαβ-dihydropiperine) (**42**)

14 mg isolated, major component of Pgst03. GC: $t_R = 67.66$ min – EIMS (m/z, % int.): 287 (M^+, 15), 202 (M^+-Piperidyl-H, 45), 204 (26), 175 (M^+-CO-Piperidyl, 40), 174 (35), 144 (20), 148 (10), 135 (100), 138 (8), 112 (5), 84 (20), 77 (22). Catalytic hydrogenated product identical with **45** previously identified [12] from the fruit.

3.4.32. N-Piperidyl-5-(3,4-methylenedioxyphenyl)-4-pentenamide (= 4.5dihydropiperine) (**43**)

Detected in Pgst03. GC: t_R 67.69 min – EIMS (m/z, % int.): 287 (M^+, 10), 202 (M^+-H-Piperidyl, 20), 174 (M^+-CO-Piperidyl, 40), 175 (28), 135 (Methylenedioxybenzyl cation, 100), 127 (10), 161 (5), 144 (5), 116 (3), 77 (9), 84 (5), 86 (10). Identified [16] from the root. After catalytic hydrogenation identical with **45**.

3.4.33. N-Piperidyl-5-(3,4-methylenedioxyphenyl)-3-pentenamide (= Δβγ-dihydropiperine) (**44**)

Present in Pgst03. GC: GC: t_R 69.13 min – EIMS (m/z, % int.): 287 (M^+, 25), 204 (M^+-Piperidyl, 100), 202 (25), 174 (42), 176 (30), 148 (43), 144 (40), 84 (48), 115 (30), 135 (20), 166 (5). New natural product, fragmentation pattern similar to that published [8].

3.4.34. N-Pyrrolidyl-5-(3,4-methylenedioxyphenyl-2,4-pentadienamide (= trichostachine) (**25**)

11 mg obtained from Pgst02. GC: $t_R = 69.93$ – EIMS (m/z, % int.): 271 (M^+, 35), 272 ($M^+ + 1$,5), 201 (M^+-Pyrrolidyl, 66), 200 (25), 202 (20), 173 (M^+-CO-Pyrrolidyl, 40), 172 (28), 171 (25), 174 (18), 143 (30), 135 (27), 115 (100), 70 (Pyrrolidyl, 5). Identical (TLC, NMR) with an authentic sample. Known component of the fruit [15].

3.4.35. N-Piperidyl-5-(3,4-methylenedioxyphenyl)-2,4-pentadienamide (= piperine) (**46**)

10mg obtained from Pgst02. GC: GC: $t_R = 70.41$ min – EIMS (m/z, % int.); 285 (M^+, 23), 284 (M^+-1,5), 201 (M^+-Piperidyl, 51), 200 (15), 202 (14), 173 (37), 172 (30), 171 (24), 174 (24), 149 (45), 143 (42), 144 (20), 135 (15), 115 (100), 89 (14), 84 (11). Identical (TLC, NMR) with an authentic. Known component of the fruit [15].

3.4.36. N-Pyrrolidyl-5-(2-methoxy-4,5-methylenedioxyphenyl) -2-pentaenamide (= Δαβ-dihydrowisanidine) (**26**)

Detected in Pgst03. GC: $t_R = 70.95$ min, EIMS (mz,% int.); 303 (M^+, 4), 205 (M^+-CO-Pyrrolidyl, 4), 166 (10), 165 (M^+-CH=CH CO-Pyrrolidyl, 100), 135 (14), 107 (9), 79 (13), 77 (17), 70 (Pyrrolidyl, 4). Data identical with that published [17] for a sample obtained from the seeds.

3.4.37. N-Isobutyl-2,4-octadecadienamide (**9**)

Detected in Pgst05. GC: $t_R = 71.23$ min – EIMS (m/z, % int.): 335 (M^+, 50), 320 (M^+-CH_3, 52), 263 (M^+-N-isobutyl, 52), 208 (M^+-$CH_3(CH_2)_8$-, 9), 194 (M^+-$CH_3(CH_2)_9$-, 10), 180 (M^+-$CH_3(CH_2)_{10}$, 20), 166 (M^+-$CH_3(CH_2)_{11}$-, 25), 152 (for-$(CH{=}CH)_2$ CON-isobutyl,70), 113 (100), 98 (85), 81 (62), 115 (40), 336 (12). Hydrogenated product gave an M^+ at m/z 339 as expected. Known constituent [15] of the fruit.

3.4.38. N-Piperidyl-5-(2-methoxy-4,5-methylenedioxyphenyl) -2-pentenamide (= Δαβ-dihydrowisanine) (**47**)

4 mg obtained in Pgst03. GC: $t_R = 71.58$ min – EIMS (m/z, % int.): 317 (M^+, 8), 84 (Piperidyl, 2), 205 (M^+-CO-Piperidyl, 3), 165 (M^+-$CH_2CH{=}CH{-}$CO-Piperidyl, 100), 166 (10), 135 (12), 115 (2), 107 (7), 79 (8), 77 (9). Fragmentation pattern of **47** and its hydrogenated product, GC: $t_R = 71.11$ min, M^+ at m/z 319 (55) identical with published data [18] for an isolate from the roots.

3.4.39. N-Piperidyl-2,4-hexadecadienamide (**40**)

Detected in Pgst 04. GC: $t_R = 71.67$ min – EIMS (m/z, % int.): 319 (M^+, 10), 234 (M^+-Piperidyl, 9), 84 (Piperidyl, 59), 192 (M^+-$CH_3(CH_2)_8$-, 89), 178 (M^+-$CH_3(CH_2)_9$-, 39), 164[$(CH{=}CH)_2$CO-Piperidyl, 75], 206 (M^+-$CH_3(CH_2)_7$-, 16), 127 (28), 112 (CO-Piperidyl, 18). Yielded a hydrogenated product GC: $t_R = 68.00$ min and major ions at m/z 323 (M^+, 2), 127 (100), 112 (36), 70 (26), 140 (23), 84 (23), 86 (15).

3.4.40. Cyclopiperstachine (**17**)

Detected in Pgst 04. GC: $t_R = 71.90$ min – EIMS (m/z, % int.): 355 (M^+, 100), 282 (M^+-N-isobutyl-H,12), 284 (5), 255 (M^+-CO−N-isobutyl, 40), 254 (42), 256 (30), 257 (4), 247 (11), 240 (11), 220 (68), 201 (10), 152 (13), 148 (30), 135 (30), 121 (42), 115 (33), 91 (44), 103 (22), 79 (22). New constituent, fragmentation identical with literature [19]. Yielded a hydrogenated product with GC: $t_R = 70$, 02min and EIMS [m/z, % int]: 357 (M^+, 95), 287 (20), 222 (100), 225 (2), 135 (85), 123 (26), 72 (4) as expected.

3.4.41. N-Piperidyl-9-octadecenamide (**41**)

Detected in Pgst03 as a minor component. GC: $t_R = 73.02$ min – EIMS (m/z, % int.): 349 (M^+, 4), 84 (Piperidyl, 25), 86 (55), 127 $(CH_3(CH_2)_7^-$, 100), 140 ($CH_3(CH_2)_7CH{=}CH$-, 45), 112 (CO-Piperidyl, 38), 154 (M^+-$(CH_2)_{6^+}$CO-Piperidyl, 5), 264 (M^+-H-Piperidyl, 2). New natural product, hydrogenated product had a GC: $t_R = 72.77$ and major ions at 351 (M^+, 2), 182 (3), 140 (26), 127 (100), 112 (42), 86 (20), 84 (Piperidyl, 25), 70 (22) as expected.

3.4.42. N-Pyrrolidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-2,4-pentadienamide (= wisanidine = okolasin = 6-methoxytrichostachine) (**30**)

Detected in Pgst03). GC: $t_R = 74.28$ min – EIMS (m/z, % int.): 301 (M^+, 100), 231 (M^+-Pyrrolidyl, 87), 230 (50), 232 (40), 233 (5), 203 (M^+-CO-Pyrrolidyl, 27) 204 (18), 202 (32), 201 (60), 187 (16), 173 (80), 172 (35), 171 (21), 151 (12), 150 (12), 145 (90), 124 (20), 115 (65), 102 (31), 70 (17). Data identical with published information [13, 20].

3.4.43. N-Piperidyl-5-(2-methoxy-4,5-methylenedioxyphenyl)-2,4-pentadienamide (= wisanine = 2-methoxy piperine) (**48**)

Obtained from Pgst04. GC: $t_R = 74.57$ min – EIMS (m/z, % int.): 315 (M^+, 100), 284 (5), 231 (M^+-Pyrrolidyl, 61), 232 (61), 230 (40), 232 (7), 215 (10), 203 (M^+-CO-Piperidyl, 24), 204 (35), 201 (35), 202 (28), 187 (32), 178 (50), 173 (82), 171 (50), 163 (21), 145 (67), 129 (25), 115 (45), 84 (26). Fragmentation identical with that given for a sample isolated from the roots [3, 4]. Hydrogenated product had a GC: $t_R = 71.11$ min and major ions at m/z 319 (M^+, 55), 234 (48), 175 (100), 165 (55), 127 (56), 86 (23), 84 (6).

3.4.44. Cyclostachine B (**29**)

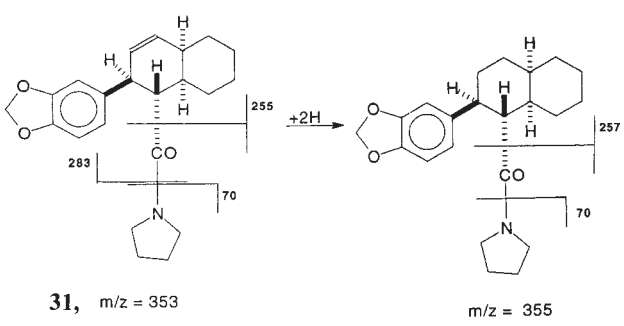
Detected also in Pgst04. GC: $t_R = 74.94$ min – EIMS (m/z, % int.): 353 (M^+, 50), 70 (Pyrrolidyl, 43), 254 (M^+-CO-Pyrrolidyl, 6), 252 (5), 323 (18), 242 (10), 228 (22), 218 (100), 135 (65), 98 (CO-Pyrrolidyl, 61), 55 (50), 72 (21), 124 (18), 150 (28), 152 (6). First report in this plant, MS identical with published data [9]. Yielded a hydrogenated product, GC: $t_R = 74.40$ min and EIMS (m/z, % int.): 355 (M^+, 97), 256 (100), 247 (27), 220 (40), 225 (27), 148 (90), 140 (83), 113 (43), 98 (47), 70 (37) as expected.

3.4.45. Cyclostachine A (**31**)

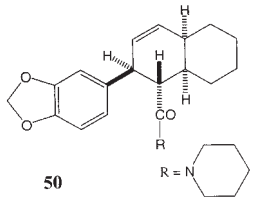
Present in Pgst04. GC: $t_R = 75.19$ min – EIMS (m/z, % int.): 353 (M^+, 100), 307 (23), 308 (16), 270 (25), 254 (M^+-CO-Pyrrolidyl, 30), 255 (19), 240 (20), 218 (58), 212 (35), 150 (31), 135 (55), 115 (52), 77 (40), 113 (40), 70 (Pyrrolidyl, 62). Yielded a hydrogenated product GC: $t_R = 74.43$ min and EIMS (m/z, % int.): 355 (M^+, 100), 257 (15), 256 (100), 246 (23), 225 (30), 220 (61), 113 (40) 135 (38), 70 (33), 98 (27), 148 (30).

3.4.46. Cycloguineense A (**50**)

Detected in Pgst04. GC: $t_R = 75.47$ min – EIMS (m/z, % int.): 367 (M^+, 100), 368 ($M^+ + 1$, 15), 337 (10), 282 (8), 254 (M^+-CO-Piperidyl, 31), 255 (11), 240 (34), 232 (76), 228 (34), 212 (12), 164 (27), 153 (12), 138 (30), 135 (46), 127 (25), 112 (65), 86 (25), 84 (Piperidyl, 36), 69 (28). This is a new natural product, the N-piperidyl derivative of cyclostachine A. The fragmentation follows the pattern postulated for cyclostachine with the piperidine moiety replacing the pyrrolidine moiety. Its hydrogenated product had GC: $t_R = 75.72$ min and major ions at m/z 369 (M^+, 75), 256 (100), 284 (15), 234 (40), 135 (62), 127 (61), 84 (13) as expected.



31, m/z = 353 m/z = 355



50

3.4.47. N-Isobutyl-2,4-eicosadienamide (**10**)

Present in Pgst05. GC: $t_R = 75.84$ min – EIMS (m/z, % int.): 363 (M^+, 62), 348 (M^+-CH_3, 38), 320 (M^+-$CH(CH_3)_2$, 12), 291 (M^+-isobutyl, 51), 263[$CH_3(CH_2)_{14}(CH{=}CH)_2$-, 5], 152[$M^+ - N{-}CH_3(CH_2)_{14}^-$, 100], 113 (95), 98 (70), 115 (70).
Data identical with published information on a sample isolated from the fruits [15, 19].

3.4.48. Cycloguineense B (**51**)

Compound was detected in Pgst04. GC: $t_R = 76.20$ min – EIMS (m/z, % int.): 367 (M^+, 100), 368 (M^++1,34), 337 (2), 324 (4), 284 (12), 254 (M^+-CO-Piperidyl, 85), 255 (29), 240 (20), 232 (60), 212 (17), 164 (19), 153 (13), 152 (13), 138 (12), 135 (65), 127 (25), 115 (25), 112 (65), 84 (Piperidyl,36), 69 (28). Isomeric to **50** eluted at GC: $t_R = 75.47$ min. A novel compound with piperidyl substituting for the pyrrolidine moiety in cyclostachine. Hydrogenated product had GC: $t_R = 75.74$ min and major ions at m/z 369 (M^+, 100), 257 (35), 256 (94), 234 (55), 127 (53) as expected.

3.4.49. N-Pyrrolidyl-7-(3,4-methylenedioxyphenyl)hepta-2,4,6-trienamide (1-piperettyl pyrrolidine) (**32**)

Detected in Pgst04. GC: $t_R = 82.31$ min – EIMS (m/z, % int.): 297 (M^+, 33), 227 (M^+-70,20), 226 (41), 225 (21), 199 (M^+-CO-Pyrrolidyl, 23), 197 (16), 196 (12), 200 (12), 169 (65), 168 (15), 141 (100), 139 (20), 115 (81), 98 (14), 70 (14). A new constituent of this plant, the hydrogenated product had a GC: $t_R = 70.97$ min and major ions at m/z, 303 (M^+, 10), 168 (25), 148 (16), 135 (53), 126 (56), 113 (100), 98 (28), 72 (27), 70 (35) behaving like a saturated aliphatic amide with strong peaks at 113 and 126 from the fission of the bond β to the carbonyl group and H_2 transfer (McLafferty rearrangement). Also prominent was the tropylium ion at m/z 135 at the expense of the acyl ion.

3.4.50. N-Piperidyl-7-(3,4-methylenedioxyphenyl)-hepta-2,4,6-trienamide (= piperettine) (**49**)

Obtained from Pgst03. GC: $t_R = 83.79$ min – EIMS (m/z, % int.): 311 (M^+, 20), 227 (M^+-84,24), 226 (72), 225 (13), 199 (M^+-CO-Piperidyl, 40), 198 (20), 200 (10), 176 (13), 169 (66), 168 (9), 141 (100), 139 (25), 135 (9), 115 (77), 112 (15), 102 (5), 86 (11). 84 (23). A new constituent, it gave a hydrogenated product GC: $t_R = 72.23$ min and major ions at m/z, 317 (M^+, 12), 232 (4), 182 (32) 148 (28), 140 (62), 135 (56), 127 (100), 112 (56), 86 (40), 84 (26), 77 (30), 70 (33), 60 (18) as expected.

3.4.51. N-Isobutyl-13-(3,4-Methylenedioxyphenyl) trideca-2,4,12-trienamide (= guineense) (**18**)

Obtained from Pgst04. GC: $t_R = 94.65$ min – EIMS (m/z, % int.): 383 (M^+, 13), 311 (M^+-72, 3), 283 (M^+-CO-N-isobutyl, 6), 282 (7), 285 (4), 261 (8), 249 (10), 248 (100), 211 (10), 201 (14), 148 (27), 149 (23), 161 (17), 135 (43), 131 (42), 103 (48), 77 (22). Previosly isolated from the fruit [16], the hydrogenated product had a GC: $t_R = 82.30$ min and major fragments at m/z 389 (M^+, 51), 359 (4), 254 (10), 135 (100), 127 (42), 115 (61), 100 (20), 60 (20) as expected.
The lignan sesamin- GC: $t_R = 80.33$ min – EIMS (m/z, % int): 354 (M^+, 18), 323 (3), 219 (6), 178 (11), 161 (23), 150 (30), 149 (100), 148 (45), 135 (26), 131 (21), 121 (20), 77 (17), 65 (13) and the sterols campesterol – GC: $t_R = 82.60$ min – EIMS (m/z, % int): 400 (M^+, 100) for $C_{28}H_{48}O$; stigmasterol – GC: $t_R = 83.81$ min – EIMS (m/z,% int): 412 (M^+, 100) for $C_{29}H_{48}O$; and sitosterol – GC: $t_R = 85.93$ min – EIMS (m/z, % int): 414 (M^+, 100) for $C_{29}H_{50}O$ were also identified from the stem.

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