



## Terpenoids from *Guarea guidonia*

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### Abstract

The volatile oil and the methanol extract from the leaves of *Guarea guidonia*, Meliaceae, were individually submitted to chromatographic separation. A sesquiterpene (2*S*\*)-eudesma-5,7-dien-2-ol, together with six known ones, were isolated from the volatile oil. The methanolic extract afforded two known and two new triterpenes (23*S*\*)-cycloart-24-ene-3 $\beta$ ,23-diol and (23*R*\*)-cycloart-24-ene-3 $\beta$ ,23-diol, besides three known sesquiterpenes, one known diterpene and two steroids. Their structures were established on the basis of spectroscopic data, mainly by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic analyses. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Guarea guidonia*; Meliaceae; Sesquiterpenes; Triterpenes

### 1. Introduction

*G. guidonia* (L.) Sleumer is a Brazilian Meliaceae species which grows in Brazil from Amazonas to Paraná States (Corrêa, 1978). This species produces a wide variety of constituents including limonoids, triterpenes, steroids, diterpenes, sesquiterpenes and coumarins (Lukacova et al., 1982; Zelnik and Rosito, 1971; Garcez et al., 1998; Lins et al., 1992). The leaves of *G. guidonia* have already been chemically investigated and different compositions have been found for specimens collected in different regions: cycloartane triterpenoids from Amazonas (Furlan et al., 1993) and São Paulo (Furlan and Lopes, 1993), clerodane diterpenoids from Rio de Janeiro (Furlan et al., 1996) and cneurobinanes diterpenoids from Pantanal region (Brochini and Roque, 2000). In continuation of our studies on *G. guidonia*, the leaves were collected in Mato Grosso do Sul. Chromatographic separations and spectroscopic analysis of the volatile oil from leaves, whose chemical composition has been described for the first time in this work, led to identification of seven sesquiterpenes (1–7), including a new derivative (2*S*\*)-eudesma-5,7-dien-2-ol (5). The methanol extract was submitted to chromatographic separation yielding three sesquiterpenes (4, 6 and 7), one diterpene (8), four tri-

terpenes (9–12), including two new ones (23*S*\*)-cycloart-24-ene-3 $\beta$ ,23-diol (11) and (23*R*\*)-cycloart-24-ene-3 $\beta$ ,23-diol (12), and two steroids (13 and 14).

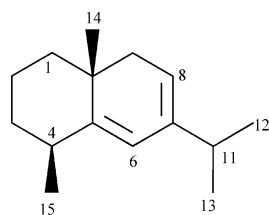
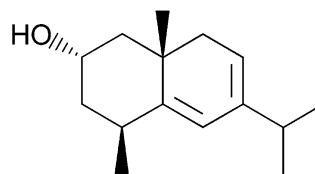
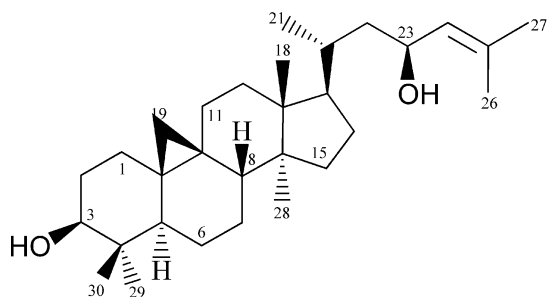
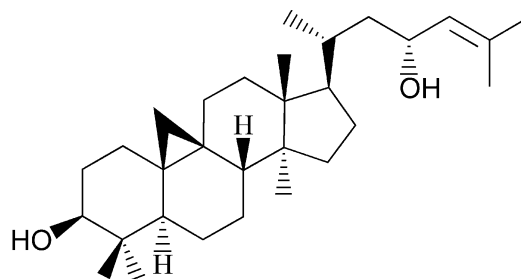
### 2. Results and discussion

The volatile oil from the leaves of *G. guidonia* was obtained by steam distillation and submitted to silica gel chromatography column. The proportion of the constituents in the crude volatile oil as well as the monitoring of the purification of the compounds were given by the FID GC chromatogram (Brochini et al., 1999). This procedure and further purification steps afforded seven pure sesquiterpenes (Table 1), corresponding to 75.6% (w/w) of the volatile oil.

The sesquiterpenes eudesma-4,11-diene (2),  $\beta$ -selinene (3), eudesm-6-en-10 $\beta$ -ol (4) and guai-6-en-10 $\beta$ -ol (7) were identified by comparison of their <sup>13</sup>C and <sup>1</sup>H NMR spectral data with those reported in the literature (Joulain and König, 1998; Maurer and Grieder, 1977; Williams et al., 1995; Fang et al., 1988; Lago et al., 2000). The comparison of the <sup>1</sup>H NMR spectral data for 1 and 6 with those reported in the literature (König et al., 1996; Bohlmann et al., 1984) indicated its structure as eudesma-5,7-diene and 5 $\alpha$ ,6 $\alpha$ ,7 $\alpha$ ,8 $\alpha$ -diepoxy-eudesmane, respectively. Their <sup>13</sup>C NMR spectral data, not previously described in the literature, are summarized in Table 2.

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**1****5****11****12**

The molecular formula  $C_{15}H_{24}O$  for **5** was established based on both the EIMS spectrum, ( $m/z$  220) and the elemental analysis. The  $^1H$  NMR spectrum displayed chemical shifts very similar to olefinic hydrogens of **1**. The  $^{13}C$  NMR spectroscopic data (Table 3) confirmed the similarity between these molecules but in the spectra of **5** an oxymethine carbon at  $\delta$  64.4 was observed instead of a methylenic group near  $\delta$  17. A  $\beta$ -desielding effect caused by the hydroxyl group at C-2 was observed for C-1 and C-3. The position of the hydroxyl group was established as  $\alpha$  equatorial because the chemical shift of C-4 is similar to that in **1**, suggesting the absence of  $\gamma$  gauche interaction between this carbon atom and the hydroxyl group. The relative configuration to hydroxyl group at C-2 was thus confirmed by comparison of the coupling constant observed to the signal at  $\delta$  4.03 (*br t*,  $J=11.4$  Hz), assigned to H-2. This value is indicative of *trans*-diaxial couplings between this

hydrogen and the adjacent H-1 and H-3, which were confirmed by the correlations observed in the  $^1H$ - $^1H$  COSY spectrum. This spectrum also showed mutual long range coupling between H-6 and H-8 as well as between H-8 and H-11. These data confirmed the structure of **5** as (2*S*\*)-eudesma-5,7-dien-2-ol. The  $^1H$  NMR data are assigned by HMQC and HMBC spectral analysis (Table 3).

The methanol extract from leaves was subjected to chromatographic separation on Sephadex LH-20 and silica gel, respectively. These separation procedures afforded three sesquiterpenes: eudesm-6-en-4 $\beta$ -ol (**4**), 5 $\alpha$ ,6 $\alpha$ ,7 $\alpha$ ,8 $\alpha$ -diepoxy-eudesmane (**6**) and guai-6-en-10 $\beta$ -ol (**7**), one diterpene: phytol (**8**), four triterpenes: 3 $\beta$ -hydroxy-cycloarta-24-en-23-one (**9**), cycloarta-24-en-3,23-dione (**10**), (2*S*\*)-cycloarta-24-en-3 $\beta$ ,23-diol (**11**) and (2*R*\*)-cycloarta-24-en-3 $\beta$ ,23-diol (**12**), and two steroids: sitosterol (**13**) and stigmasterol (**14**).

Table 1  
Retention time and proportion in the crude volatile oil of the sesquiterpenes **1**–**7**

	Sesquiterpene	$RR_t$ (sec)	$RF^a$	% In the crude oil
<b>1</b>	Eudesma-5,7-diene	415	1006	19.2
<b>2</b>	Eudesma-4,11-diene	497	1205	6.1
<b>3</b>	$\beta$ -Selinene	501	1215	5.8
<b>4</b>	Eudesm-6-en-4 $\beta$ -ol	672	1630	21.0
<b>7</b>	Guai-6-en-10 $\beta$ -ol	682	1654	21.0
<b>5</b>	(2 <i>S</i> *)-Eudesma-5,7-dien-2-ol	690	1672	1.6
<b>6</b>	5 $\alpha$ ,6 $\alpha$ ,7 $\alpha$ ,8 $\alpha$ -Diepoxy-eudesmane	870	2110	0.9
			TOTAL	75.6

<sup>a</sup>  $RF$ : retention index in DB-50.

Table 2  
<sup>13</sup>C NMR spectral data for sesquiterpenes **1** and **6** (50 and 200 MHz,  $\delta$ , CDCl<sub>3</sub>)

Position	<b>1</b>	<b>6</b>
1	41.7 <sup>a</sup>	38.1
2	17.6	17.0
3	31.6	29.9
4	32.9	32.1
5	149.6	53.2
6	114.5	56.1
7	141.4	no
8	120.9	51.2
9	41.4 <sup>a</sup>	38.5
10	34.2	34.6
11	35.6	37.4
12	21.4	18.1 <sup>a</sup>
13	21.8	22.2
14	23.6 <sup>a</sup>	17.5
15	24.5 <sup>a</sup>	18.0 <sup>a</sup>

<sup>a</sup> Value may be reversed in the columns.

Table 3  
 NMR spectral data for sesquiterpene **5** (50 and 200 MHz,  $\delta$ , CDCl<sub>3</sub>)

Position	<sup>13</sup> C	<sup>1</sup> H (multiplicity, J/Hz)
1	50.2	1.18 (m)
2	64.4	4.03 (br t, 11.4)
3	40.8	1.44 (m)
4	32.9	2.69 (m)
5	147.2	–
6	114.3	5.59 (s)
7	141.5	–
8	121.3	5.23 (m)
9	41.5	1.80 (m)
10	No	–
11	36.2	2.24 (m)
12	21.3	0.95 <sup>a</sup> (s)
13	21.7	0.92 <sup>a</sup> (s)
14	24.3 <sup>a</sup>	0.96 (s)
15	25.0 <sup>a</sup>	1.13 (d, 7.6)

<sup>a</sup> Value may be reversed in the columns.

Compounds **8**, **9**, **10**, **13** and **14**, as well as the sesquiterpenes **4**, **6** and **7**, previously characterized in the volatile oil, were identified by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic analyses (Rahman and Ahmad, 1992; Furlan et al., 1993; Lago et al., 2000).

The <sup>1</sup>H NMR spectrum of **11** had two doublets at  $\delta$  0.32 ( $J=4.2$  Hz) and at  $\delta$  0.55 ( $J=4.0$  Hz), one doublet at  $\delta$  0.94 ( $J=6.5$  Hz) and six singlets at  $\delta$  0.96, 0.94, 0.89, 0.81, 1.74 and 1.71, assignable to seven methyl groups. These data associated with the occurrence of one *dd* at  $\delta$  3.28 ( $J=11.5$  and 4.4 Hz), are characteristic of a cycloartan-3 $\beta$ -ol type triterpene. The presence of one olefinic hydrogen at  $\delta$  5.10 (*dt*,  $J=9.0$  and 1.3 Hz) and one allylic oxymethine hydrogen at  $\delta$  4.46 (*td*,  $J=9.3$  and 4.2 Hz), positioned the double bond at C-24 and the hydroxyl group at C-23. The <sup>13</sup>C NMR spec-

trum (Table 4) indicated the presence of thirty carbon atoms, including two oxymethine groups at  $\delta$  78.9 and  $\delta$  67.3, which were assigned to C-3 and C-23 respectively. The olefine carbons atoms C-24 and C-25 were observed at  $\delta$  128.4 (CH) and  $\delta$  135.6 (C). These data, together with HMBC correlations, confirmed the occurrence of a cycloartan-3 $\beta$ -ol triterpene which had the following substructure to the side chain  $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}(\text{OH})\text{CH}=\text{C}(\text{CH}_3)_2$ . The relative configuration of C-23 was defined by comparison of the spectrometric data to those of tirucalane triterpenoids in *Dysoxylum macranthum* (Mohamad et al., 1999) and *D. variable* (Liu et al., 2001), which has the same side chain as **11**. The similarity between the signals of C-22, C-23, C-24, C-25, C-26 and C-27 of these tirucalanes and of **11**, indicated the same spatial relationship to substituents of the side chain. Therefore, its structure was defined as (23*S*\*)-cycloarta-24-en-3 $\beta$ ,23-diol. The hydrogen bearing carbon signals were assigned by HMQC spectrum (Table 4).

The <sup>1</sup>H and <sup>13</sup>C NMR spectra (BBD and DEPT 135°) of **12** showed similar chemical shifts and same multiplicity to all carbon atoms as in **11** (Table 4) indicating the same planar structure for both molecules, confirmed by the superimposable IR spectrum. The EIMS data of both triterpenes showed a signal at  $m/z$  424 [ $\text{M}-\text{H}_2\text{O}$ ]<sup>+</sup> and similar fragment ions. However, the <sup>13</sup>C NMR spectra of **12** showed sp<sup>2</sup> carbons at  $\delta$  129.1 (CH) and  $\delta$  133.8 (C) and one oxymethine carbon atom at  $\delta$  66.1 (C), which were assigned to C-24, C-25 and C-23, respectively. The value of resonances of the side chain carbons of **11** and **12** are similar to those observed to C-23 epimeric mixture of 23-hydroxy-cycloarta-24-en-3-ones, isolated from *G. trichilioides* (Furlan et al., 1993). These results suggested the opposite configuration for C-23 in **12** relative to that defined for **11**. Therefore **12** was defined as (23*R*\*)-cycloarta-24-en-3 $\beta$ ,23-diol (Table 4).

Several sesquiterpenes, diterpenes and triterpenes have been identified in *G. guidonia*, but limonoids (meliacins), which are produced by the oxidative degradation of the side chain from euphol/tirucalol type triterpenoids (Goodwin and Mercer, 1983), have not been detected yet in the leaves. However, the wood bark of *G. guidonia* collected in Mato Grosso do Sul did not contain cycloartane derivatives, although one limonoid and several sesquiterpenes were isolated (Garcez et al., 1998). In *G. macrophylla* several cycloartane derivatives were isolated from leaves, but no limonoids nor monoterpenes in the volatile oil have been detected (Lago et al., 2000; Lago and Roque, 2002a).

Since several sesquiterpenes and no monoterpenes have been identified in the volatile oil from the stem bark of *G. guidonia* (Núñez and Roque, 1998) and *G. cedrata* (Menuet et al., 1995) and from the leaves, stem bark and fruits of *G. macrophylla* (Lago and Roque, 2002b; Lago et al., 2002a,b), this might be characteristic of the *Guarea* species.

Table 4  
<sup>13</sup>C and <sup>1</sup>H NMR spectral data for triterpenes **11** and **12** (500 and 125 MHz,  $\delta$ , CDCl<sub>3</sub>)

Position	<sup>13</sup> C		<sup>1</sup> H (multiplicity, J/Hz)	
	11	12	11	12
1	32.0	32.0	1.56 (m)/1.21 (m)	1.55 (m)/1.22 (m)
2	30.3	30.4	1.75 (m)/1.56 (m)	1.75 (m)/1.56 (m)
3	78.9	78.9	3.28 (dd, 11.5, 4.4)	3.27 (dd, 11.1, 4.4)
4	40.5	40.5	—	—
5	47.1	47.1	1.31 (m)	1.28 (m)
6	21.1	21.1	1.58 (m)	1.60 (m)
7	28.4	28.3	1.95 (m)	1.92 (m)
8	47.9	47.9	1.51 (m)	1.52 (m)
9	20.0	20.0	—	—
10	25.9	25.7	—	—
11	26.0	26.0	1.31 (m)/1.09 (m)	1.32 (m)/1.09 (m)
12	35.5	35.5	1.29 (m)	1.30 (m)
13	45.3	45.4	—	—
14	48.9	48.9	—	—
15	32.9	32.7	1.61 (m)	1.50 (m)
16	26.4	26.5	1.98 (m)	1.98 (m)
17	52.9	52.9	1.62 (m)	1.56 (m)
18	18.0	18.1	0.94 (s)	0.99 (s)
19	29.9	29.9	0.55 (d, 4.0)/0.32 (d, 4.0)	0.55 (d, 4.1)/0.33 (d, 4.1)
20	33.5	33.0	1.30 (m)	1.61 (m)
21	19.1	18.1	0.94 (d, 6.5)	0.89 (d, 6.5)
22	44.5	44.5	1.61 (m)/1.35 (m)	1.62 (m)/1.04 (m)
23	67.3	66.1	4.46 (td, 9.3, 4.2)	4.47 (td, 9.0, 3.0)
24	128.4	129.1	5.10 (dt, 9.0, 1.3)	5.20 (dt, 8.7, 1.3)
25	135.6	133.8	—	—
26	25.4	25.4	1.74 (s)	1.71 (s)
27	18.3	18.3	1.71 (s)	1.68 (s)
28	19.3	19.3	0.89 (s)	0.88 (s)
29	14.0	14.0	0.81 (s)	0.80 (s)
30	26.1	26.1	0.96 (s)	0.96 (s)

### 3. Experimental

#### 3.1. Plant material

The leaves of *Guarea guidonia* (L.) Sleumer were collected, from the same specimen, on November 1996 (volatile oil) and on March 1998 (methanol extract) in Campo Grande, Mato Grosso do Sul State, Brazil. The plant material was identified by Prof. Humberto Barreiros, from the Jardim Botânico do Rio de Janeiro, RJ, Brazil. A voucher specimen (number 1870) is deposited in the herbarium of the Universidade Federal do Mato Grosso do Sul, MS, Brazil.

#### 3.2. General

Silica gel 60 was used for chromatography: 63–200  $\mu$ m for CC, 40–63  $\mu$ m for flash chromatography and GF<sub>254</sub> (5–40  $\mu$ m) for prep. TLC. Sephadex LH-20 (Sigma) was used in exclusion chromatography. Optical rotations were measured in a digital polarimeter JASCO DIP-370 (Na filter,  $\lambda$  = 588 nm). IR spectra were obtained as KBr pellets in a Perkin-Elmer Infrared Spectrometer model 1750. NMR spectra were recorded at 125 or 50 MHz for

<sup>13</sup>C and 500 or 200 MHz for <sup>1</sup>H (Bruker DRX-500 or Bruker-AC200) using CDCl<sub>3</sub> as solvent and int. standard. The GC–MS analysis was performed at 70 eV in a INCOS 50 Finnigan-Mat-quadrupole spectrometer, using a capillary column (DB-50) coated with crosslinked methyl silicone gum (25 m×0.200 mm i.d., film thickness 0.33 $\mu$ m). The temp. program was 100 °C isothermal for 1 min, then 100–250 °C at 10 °C min<sup>−1</sup>, and isothermal at 250 °C for 20 min. The temp. of injection and detection were 230 and 280 °C respectively. GC–FID chromatograms were obtained in HP 5890 series II. The temp. program of GC analysis was 100 °C isothermal for 2 min, 100–180 °C at 5 °C min<sup>−1</sup>, isothermal at 180 °C for 2 min, 180–250 °C at 10 °C min<sup>−1</sup>, then isothermal at 250 °C for 5 min. The temp. of injection and detection (flame ionization) were 180 and 220 °C respectively. A capillary column (DB-50) coated with 5% PhMe silicone (30 m×0.32 mm i.d., film thickness 0.25  $\mu$ m) was used.

#### 3.3. Extraction and isolation of the constituents from the volatile oil

The fresh leaves of *G. guidonia* (600 g) were steam distilled using a Clevenger-type apparatus to give the

crude volatile oil (290 mg, 0.05%). The oil was submitted to flash chromatography over silica gel eluted with  $\text{CH}_2\text{Cl}_2$  affording **1** (fraction 1, 12 mg) and **4** (fraction 5, 10 mg). Fraction 2 was submitted to further purification on silica gel coated with  $\text{AgNO}_3$  (15%), eluted with  $\text{CH}_2\text{Cl}_2$ , yielding **2** (21 mg) and **3** (17 mg). Fraction 8 gave **7** (20 mg) after prep. TLC eluted with  $\text{CH}_2\text{Cl}_2$ . Fraction 9 was also submitted to prep. TLC using  $\text{CH}_2\text{Cl}_2$ :MeOH (99:1) as eluent. This procedure afforded pure **5** (10 mg), **6** (9 mg) and **7** (11 mg).

### 3.4. (2*S*\*)-Eudesma-5,7-dien-2-ol (**5**)

Yellow oil.  $[\alpha]_D^{25} + 6$  ( $\text{CHCl}_3$ ;  $c$  0.25). IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3406, 2964, 1712, 1665, 1258, 1167, 1038, 967.  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectroscopic data are given in Table 3. EIMS (70 eV)  $m/z$  (rel. int.): 220 (33), 202 (51), 161 (100), 145 (58), 105 (43), 55 (17). Found C, 81.82%; H 11.07%, requires: C, 81.76%; H 10.89%.

### 3.5. Extraction and isolation of the constituents from methanol extract

The air dried leaves of *G. guidonia* (180 g) were extracted with MeOH three times. The crude extract (14 g) was applied to a Sephadex LH-20 column eluted with hexane:  $\text{CH}_2\text{Cl}_2$  (1:4),  $\text{CH}_2\text{Cl}_2$ :acetone (3:2) and  $\text{CH}_2\text{Cl}_2$ :acetone (1:4) to yield three fractions after TLC analysis. Fraction 1 consisted of waxy material and fraction 3 was formed by pigments. Fraction 2 (2 g) was subjected to CC on silica gel eluted with increasing amounts of EtOAc in hexane and EtOAc–MeOH to yield ten fractions. Fraction 3 was composed of a mixture of **4** and **6** (110 mg). Fraction 4 (132 mg), after CC on silica gel eluted with hexane– $\text{CH}_2\text{Cl}_2$  and  $\text{CH}_2\text{Cl}_2$  with increasing amounts of MeOH yielded **7** (29 mg) and **8** (13 mg). Fraction 5 (109 mg) was subjected to CC on silica gel eluted with gradient of hexane: $\text{CH}_2\text{Cl}_2$ :MeOH to afford **7** (8 mg) and mixtures of **7** and **13** (14 mg), **13** and **14** (14 mg) and of **9** and **11** (63 mg). Fraction 6 (230 mg) was separated by CC on silica gel, eluted with  $\text{CH}_2\text{Cl}_2$  with increasing amounts of EtOAc to give **11** (24 mg) and **12** (49 mg).

### 3.6. (2*S*\*)-Cycloart-24-ene-3 $\beta$ ,23-diol (**11**)

Colorless amorphous solid.  $[\alpha]_D^{24} + 23$  ( $\text{CHCl}_3$ ,  $c$  0.15). IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3426, 2933, 2869, 1708, 1459, 1378, 1049, 1024, 1005, 890, 756, 603.  $^{13}\text{C}$  R and  $^1\text{H}$  NM spectral data are given in Table 4. EIMS  $m/z$  (rel. int.): 442  $[\text{M}]^+$  (not observed), 424  $[\text{M}-\text{H}_2\text{O}]^+$  (6), 406 (8), 391 (11), 363 (10), 313 (9), 295 (5), 241 (8), 227 (6), 203 (12), 187 (16), 175 (20), 161 (28), 159 (25), 135 (29), 119 (42), 109 (52), 95 (61), 81 (100), 67 (65), 55 (86). Found C, 81.47%; H 11.47%, requires C, 81.39%; H 11.38%.

### 3.7. (2*R*\*)-Cycloart-24-ene-3 $\beta$ ,23-diol (**12**)

Colorless amorphous solid.  $[\alpha]_D^{24} + 35$  ( $\text{CHCl}_3$ ,  $c$  0.21). IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3399, 2925, 2867, 1710, 1462, 1378, 1048, 1022, 1005, 889, 756, 592.  $^{13}\text{C}$  NMR and  $^1\text{H}$  are given in Table 5. EIMS  $m/z$  (rel. int.): 442  $[\text{M}]^+$  (not observed), 424  $[\text{M}-\text{H}_2\text{O}]^+$  (5), 406 (7), 391 (10), 363 (7), 313 (10), 295 (5), 269 (7), 241 (6), 229 (7), 203 (10), 187 (16), 175 (15), 161 (20), 133 (24), 119 (36), 109 (49), 95 (53), 81 (100), 67 (60), 55 (73). Found C, 81.46%; H 11.45%, requires C, 81.39%; H 11.38%.

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