Terpenoids from *Cnidoscolus phyllacanthus* Pax et Hoff

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Investigação química de *Cnidoscolus phyllacanthus* conduziu ao isolamento de três novos terpenóides: um bis-*nor*-diterpeno, filacantona (**1**), e dois triterpenos, 3β-O-cinamoil-lupeol (**2**) e 3β-O-dihidrocinamoil-lupeol, (**3**). A elucidação estrutural destas substâncias foi realizada com base na análise de dados espectrométricos, inclusive experiências bidimensionais (2D) de RMN.

Chemical investigation of *Cnidoscolus phyllacanthus* has led to the isolation of three new terpenoids: a bis-nor-diterpene, phyllacanthone (**1**), and two triterpenes 3β-O-cinnamoyl-lupeol (2) and 3β-O-dihydrocinnamoyl lupeol (**3**). Structure determinations have been done by spectral analysis, including 2D-NMR methods and by comparison with model compounds from the literature.

Key Words: Cnidoscolus phyllacanthus; *Euphorbiaceae; bis*-nor-*diterpene; triterpenes.*

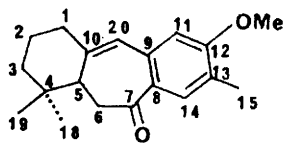
Introduction

Cnidoscolus phyllacanthus Pax et Hoff, (Euphorbiaceae), popularly known as "favela" or "faveleira", is well dispersed throughout the "caatinga", a characteristic flora of Northeastern Brazil. This species is well known by the peasants for its stinging nettles what cause severe pain and wounds that heal with difficulty. However, its dried leaves and bark are used as fodder for donkeys and pigs and the seeds as feed for chicken during starving seasons[1].

This paper is concerned with the isolation and structure elucidation of three new terpenoids: 7-oxo-12-methoxy-16, 17-bis-*nor*-9(10→20)-*abeo*-abieta-10 (20),8,11,13-tetraene, which has been given the trivial name phyllacanthone (1), 3β-O-cinnamoyl-lupeol,(2), and 3β-O-dihydrocinnamoyl-lupeol (3). Phyllacanthone (1), is the first member of a bis-*nor* terpenoid class which possesses a isopisiferin type skeleton[2,3].

Results and Discussion

The hexane extract of the powdered trunk bark of *Cnidoscolus phyllacanthus* was chromatographed over silica gel to yield four fractions by elution with hexane followed by chloroform, ethyl acetate and finally methanol. The hexane fractions showed two major spots on TLC and afforded (1), (2) and (3) after rechromatography over a silica gel column. The IR spectrum (neat) of (1) showed the presence of a conjugated carbonyl (ν = 1650 cm^{-1}) and an aromatic ring (ν = 1595 and 1495 cm^{-1}). The molecular formula of this compound was determined as $C_{19}H_{24}O_2$ based on the HRMS (M^+284.1789, found 284.1900) and on the 24 protons and 19 carbon signals observed in the ^1H and ^{13}C spectra, respectively (Table 1). Further analysis of the ^1H NMR spectrum (Table 1) revealed signals for three methyl groups, two attached to quarternary sp^3 carbons [δ 1.12(s) and 0.76 (s)] and one to a sp^2 carbon [δ 2.18 (s)]; one methoxy group (δ 3.87 (s); three hydrogen atoms on sp^2 carbons [δ 6.59 (s), *ortho* to



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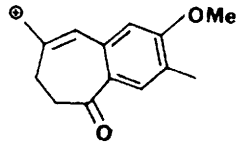
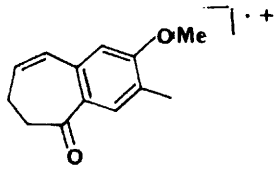
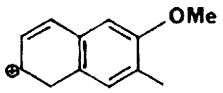


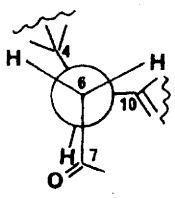
1a, m/z 215



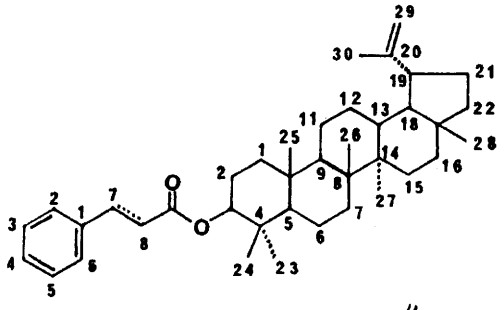
1b, m/z 202



1c, m/z 173

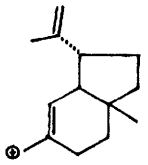


1d

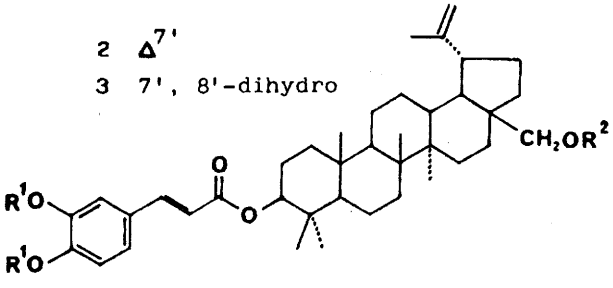


2 $\Delta^{7'}$

3 7', 8'-dihydro

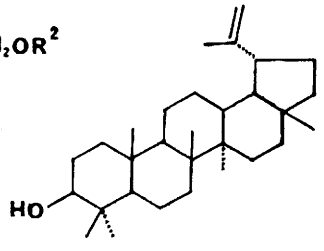


3a, m/z 189



4 $R^1=R^2=H$

4a $R^1=R^2=Ac$



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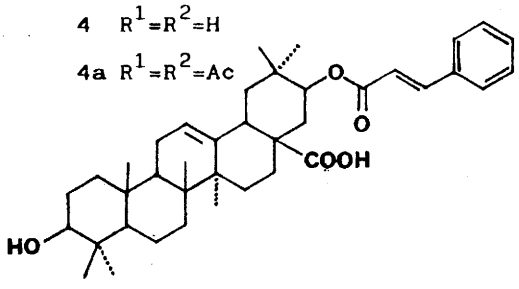

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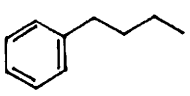

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Table 1. *Proton (300 MHz) and Carbon-13 (75MHz)* chemical shifts (δ, $CDCl_3$) assignments. Homonuclear (⟷) and long-range heteronuclear correlations (C⟷H) observed for bis-nor-diterpenoid (1), by J modulation for 12.0 and 7.5 Hz.*

C#	δC	J_{CH}=12.0Hz	J_{CH}=7.5Hz	δH	^1H-^1H COSY
1	40.2			2.3-2.5	
2	25.1			1.98 -1.6	
3	43.0			1.5 -1.4	
4	38.4			-	
5	51.3			2.4	
6	42.5			3.0	
				3.0	
7	201.3			-	
8	129.7			-	
9	136.3			-	
10	147.7			-	
11	112.9			6.6	
12	160.9			-	
13	124.7			-	
14	131.2			7.6	
15	15.5			2.2	
18	28.9			1.1	
19	20.7			0.8	
20	125.5			6.3	
OMe	55.4			3.9	

* *Hydrogenation pattern for carbon signals was deduced either by APT or DEPT.*

an oxygen atom; δ 7.62(s), *peri* to a carbonyl function and δ 6.29 (br,s), a vinyl hydrogen], along with other signals attributed to hydrogen atoms attached to sp^3 carbon (Table 1).This interpretation was facilitated by the homonuclear (2D-^1H -^1H COSY) and heteronuclear (2D - ^1H -^{13}C COSY; modulated with $^1J_{CH}$), shift correlated NMR spectra (Table 1). The number of attached protons for each carbon signal observed in the ^{13}C NMR spectrum was deduced by comparative analysis of the PND, DEPT and APT spectra (Table 1). These data, in combination with the ^1H NMR spectrum, allowed expansion of the molecular formula $C_{19}H_{24}O_2$ to $(C{=}O(C{=}C)(C{=}CH)_3(C)(CH)(CH_2)_4(CH_3)_3(OCH_3)$. The presence of a methoxy group allowed establishment the molecular formula $C_{18}H_{21}O$ for the basic skeleton and to classify the natural products a bis-*nor*-diterpenoid with 8 degrees of unsaturation (rings and/or multiple bonds). The presence of an acetophenone moiety [νC=O, 1650; νC=C 1595, 1490 cm^{-1} (aromatic ring)] was recognized by the ^1H chemical shift [δ 7.62 (s,H-14) and 6.59(s,H-11)], corresponding to two protons *para*-related as well as the ^{13}C chemical shifts at [δ 129.69 (s,C-8), 136.28(s,C-9), 112.87 (d,CH-11), 160.87 (s,C-12), 124.66 (s,C=13) and 131.19 (d,CH-14).

Long-range heteronuclear shift correlations (^{13}C-^1H COSY) with the mixing period adjusted to an average value of $^2J_{CH}$ and $^3J_{CH}$ (J=12.0 and J=7.0 Hz), sequence denoted as COLOC (correlation via long-range coupling)[4], were utilized to confirm the substitution pattern of the aromatic ring and locate the substituents on this system, to confirm the assignments on the ^1H and ^{13}C chemical shifts, and to define other carbon-carbon connectivities (Table 1).Thus, the presence of the methoxy group at carbon 12 and a methyl group at C-13 was defined by the spin-spin interaction (Table 1) of C-12 (δ 160.87) with H-14 (δ 7.62), H-15 (δ 2.18), the protons of the OMe group (δ 3.87) and H-11 (δ 6.59), C-13 (δ 124.66) with H-11 (δ 6.59) and H-15(δ 2,18), and C-14 (δ 131.19) with H-15 (d 2.18). The location of the methoxy and methyl groups at C-12 and C-13, respectively was used as diagnostic information for the classification (1) as a 16, 17-bis-*nor*-abietane diterpene. Suggestions about the secondary

biomodification of the isopropyl group include elimination of Me-16 and Me-17 by oxidative demethylation (as formic acid or decarboxylation)[4] and formation of the CH_3-15. Additional analysis of the ^1H -^{13}C 2D heteronuclear correlation via long-range coupling spectrum (modulated by J=12.0 and J=7.0 Hz) allowed the definition of the carbon sequences through the interactions pointed out in Table 1 by the arrows (← →). These data were used to deduce the structure (1) unambiguously. The homonuclear (^1H-^1H-COSY) 2D shift-correlated NMR spectrum of (1) confirmed this deduction by nothing the spin-spin interaction between H-11 and H-20, H-14 and H-15, H-5 and H-6. It is interesting to note that the natural occurrence of (1) as a modified bis-nor-diterpenoid can be explained by the cleavage of the bond C_9-C_{10} (9,10-*seco*) and biogenetic cyclization for the formation of C_9-C_{20} bond. This modification can be indicated by the expression "9(10→20)-*abeo*"[5] . Thus, the structure of the new diterpene isolated from *Cnidoscolus phyllacanthus* was established as 12-methoxy-9(10→20)-*abeo*-16,17-bis-*nor*- abieta-8, 10(20),11,13-tetraen-7-one(1). Analogous diter- penoids maintaining the unmodified isopropyl group have been isolated from *Chamaecyparis pissifero*[9], *Slavia fallotaeflora* and *S.anaetosomane*[5]. The mass spectrum of (1) showed the molecular ion peak at m/z 284 (92%, M^+), and major peaks at 215 (88%), 202 (100%) and 173 (92%). Peaks showing m/z 215, 202 and 173 can be represent fragments 1a, 1b and 1c, respectively. Finally, our attention was directed to the small vicinal coupling constants revealed by H-6α and H-6β signals [δ 3.01 (dd, Jgem=13.1 and J_{vic}=1.3), 3.03 δ (dd, J_{gem}=13.1 and J=0.7 Hz)] Coupling of these protons with H-5 [δ 2.36(m)] was cleary confirmed by cross peaks in the ^1H-^1H COSY spectrum. Examination of Dreiding models demonstrated than in a conformation maintaining the coplanarity of the carbonyl group and the aromatic ring [δ 7.62 (H-14)], the methine proton H-5 assumes a trans relationship to both vicinal H-6α and H-6β as shown in 1d (Newman projection). These observed vicinal coupling constants (J=1.3 and 0.7 Hz) can be predicted by the Karplus Conroy relationship (dihedral angles involving H-5, H-6α and H-6β).

Table 2. *^{13}C NMR data (75 mHz, $CDCl_3$, TMS as internal standard) of 3β-O-cinnamyl 2 and 3b-O-dihidrocinnamil 3 and comparison with the models **4a, 5, 6** and 7 chemcial shifts (δ) are expressed in ppm.*

C	2 / 3	4a	5	C	2	3	4a	6	7
1	38.4 / 38.4	38.3	38.7	1'	134.6	140.6		134.3	143.3
2	23.7 / 23.9	23.6	27.4	2', 6'	128.3	128.0		127.9	129.0
3	81.0	81.0	78.9	3', 5'	128.5	128.9		128.7	128.2
4	37.9	37.9	38.3	4'	130.1	126.2		130.0	125.7
5	55.4 / 55.5	55.3	55.3	7'	144.3	31.1	142.1	144.3	
6	18.2	18.0	18.3	8'	118.9	36.2	119.9	118.4	
7	34.3	34.3	34.2	9'	166.8	172.7	166.2	166.3	
8	40.9	40.8	40.8						
9	50.4	50.2	50.4						
10	37.1	36.9	37.1						
11	20.9	20.7	20.9						
12	25.1	25.0	25.1						
13	38.1	37.4	38.0						
14	42.3	42.5	42.8						
15	27.5		27.4						
16	35.6		35.5						
17	43.0		43.0						
18	48.3		48.2						
19	48.0	47.7	47.9						
20	150.9	149.9							
21	29.9								
22	40.0								
23	27.9 / 28.1	27.8							
24	16.5 / 16.7	16.5							
25	16.2 / 16.2	16.0							
26	16.0	15.9							
27	14.5	14.6							
28	18.0								
29	109.4	109.7							
30	19.3	18.9							

* *The multiplicity of carbon signals was deduced using APT and DEPT techniques. Only the chemical shift data useful to comparative analysis are described.*

The presence of a lupeolyl moiety in the components of a mixture of (2) and (3) was recognized by chemical shifts and multiplicity of all ^{13}C NMR signals, deduced by comparative analysis of the proton noise decoupled (PND), APT and DEPT spectra (Table 2). The ^{1}H NMR displayed three broad singlet signals for two ethylenic protons (δ 4.70 and 4.58) and one methyl group (δ 1.69) in a 1,1-disubstituted double bond (ν max 1640 and 890 cm^{-1}), along with signals attribute to six tertiary methyl groups between δ 1.42 and 0.79 (all singlets), and two carbinolic protons at δ 4.50 (dd, J=10.2, J=4.5 Hz) and 4.63 (dd, J=10.3, J=4.5 Hz) showing different intensities (Table 2). The ^{1}H and ^{13}C assignments of these moieties was corroborated by application of the usual chemical shift parameters[6], comparison to model compounds 4a[7] and 5[8] and the observed multiplicity of signals in the DEPT-^{13}C NMr spectrum (Table1). The chemical shift of H-3 [2(minor component): δ 4.63; 3: δ 4.50] and carbon atoms C-3 (δ (δ 81.04), C-2 [2: δ 23.71; 3; δ 23.85] and C-4 (δ 37.83) were compared to those of triterpene 4a, this comparison indicated the presence of an ester function at C-3 [2: δ 166.80, ν max 1712 cm^{-1}; 3: δ 172.68, ν max 1740 cm^{-1}].Thus, the two new triterpenes (2) and (3) present in the mixture were both esters and the site of esterification was established at C-3 of the A ring of the basic skeleton of lupeol. The recognition of the presence of this basic skeleton in the two natural esters (2,3) pointed to the esterification of the hydroxyl function at C-3 with different acyl groups. The IR spectrum of this mixture exhibited absorption bands at 1712 (C=0), ester) as a broad band, 1629 (C=CH), and 1510, 1600 cm^{-1} (aromatic ring). Further analysis of the ^{1}H NMR spectrum indicated a cinnamyl group 2 in which was indicated by signals at δ 7.68 (d,J=16.5 Hz, H-7), 7.6-7.5 (m,H-2',6'), 7.4-7.3 (m, H-3',5',5') and 6.45 (d, J=16.5 Hz, H-8'). The coupling constant (J=16.5 Hz) observed for the signals of H-7'and H-8' characterized this group as a *E*-cinnamyl moiety. The presence of this group was confirmed by the ^{13}C NMR spectrum [δ 166.80(s,C-9'), 144.24 (d,C-7'), 134.3 (s,C-1'), 130.11 (d,C'4'), 128.45 (d,C-3',5'), 128.27 (d,C-2',6'), 118.91 (d,C-8')] and was supported by a comparative analysis of a *E*-cinnamyl group in the model compound 6[9] (Table 2).

The 7',8'-dihydrocinnamyl group corresponding to the major component 3 was also indicated by ^{1}H[δ 7.3 - 7.1 (m, H-2'to H-6'), 2.98 (t, J=7.8 Hz, H-7'), 2.69 (t, J=7.8 Hz, H-8') and ^{13}C [δ 172.68 (s, C-9'), 140.59 (s, C-1') 128.84 (d, C-3', 5'), 128.04 (d, C-2',6'), 126.19 (d, C-4'), 36.24 (t, C-80'), 31.12 (t, C-7')] NMR analysis (Table 2). The ^{13}C NMR assignments of this group included the comparison to model compound 7 and the application of the usual chemical shift parameters[6]. The assignment of an equatorial-position for the *E*-cinnamoyloxy and 7',8'-dihydrocinnamoloxy groups at C-3 was deduced from the chemical shifts of carbons 1 to 5, 23 and 24[9] and by the presence of two signals at δ 4.63(2) and 4.5 (3) as double doublets ($J_{ax,ax}$=10.3 Hz and J_{ae}= 4.5 Hz). The intensities (revealed integration), of the signals at δ 6.45 and 2.98 which corresponded to proton H-8'and the methylenic protons at C-7 of 2 and 3 respectively, were used to estimate the relative percentages of these compounds in the mixture: 33.33% of 2 and 66.67% of 3. The EIMS showed a peak at m/z 408 ($C_{30}H_{48}$) generated from molecular ions of 2[$C_{39}H_{56}O_2$, M^{+}556 (absent)] and 3[$C_{39}H_{58}O_2$, M^{+} 558 (absent)] through a McLafferty rearrangement and elimination of cinnamic ($C_9H_8O_2$) and dihydrocinnamic ($C_9H_{10}O_2$) acids respectively. Other diagnostic peaks were observed at m/z 189 (43%), 150 (13%), 148 (14%), 133 (37%), 131 (21%) and 91 (100%). The peak at m/z 189 can be attributed to fragment 3a as representative of the lupeoly moiety[10]. The peaks at m/z 150 ($C_9H_{10}O_2^{\cdot+}$), 133 [$C_9H_9O^{+}$, 150-17 ($C_9H_{10}O_2^{\cdot+}$ - ·OH)] and 91 (tropilium ion =$C_7H_7^{+}$) are consistent with the presence of dihydrocinnamoloxy group in 3. Analogously, the peaks at m/z 148 ($C_9H_8O_2^{\cdot+}$) and 131[($C_9H_7O^{+}$, 148-17 ($C_9H_8O_2^{\cdot+}$ -·OH)] can be rationalized as fragments produced by existence of the cinnamoloxy in 2.

Thus, on the basis of spectral data the structures of three new terpenoids isolated from *Cnidoscolus phyllacanthus* were established as 12-methoxy-9(10-20)-*abeo*-16,17-bis*nor*-abieta-8,10(20), 11, 13-tetraen-7-one (1) 3β-O-cinna-moyl-lupeol(2) and 3β-O-dihydrocinnamoyl-lupeol (3).

Experimental

General Experimental Procedures: m.p. were determined using a Kofler hot stage instrument and are not corrected. IR spectra were taken on a Perkin Elmer 281 Spectrometer U.V. spectra were recorded on a Beckman spectrophotometer Acta III in ethanol solution. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, using TMS as internal standard employing a Varian VXR-300 (^{1}H:300 MHz; ^{13}C; 75 MHz) spectrometer. Low resolution mass spectra were recorded on a Finnigan 3200 GC\MS instrument operating at 70 *eV*.

Plant Material: Barks of *Cnidoscolus phyllacanthus*, Euphorbiaceae were collected in Lajes, Rio Grande do Norte State, Brasil, and identified by Professor Afranio G. Fernandes. A voucher specimen (n° 14923) was deposited in the hebarium (Herbário Prisco Bezerra) of the Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, Ceará, Brasil.

Extraction and isolation of constituents. The powdered bark (4 kg) was submitted to cold percolation using hexane followed by ethanol. The crude hexane extract (98g), obtained after concentration of solution under vacuum, was coarsely chromatographed over silica gel using hexane, $CHCl_3$, EtOAc and MeOH to provide four fractions. The fraction eluted with hexane was rechromatographed over silica gel to yield a fluorescent material whose final purification was achieved by preparative TLC using a mixture of hexane-$CHCl_3$ (1:1) to yield 1 (95 mg). From the same hexane fraction a second material was also obtained as a solid material (250 mg), the final purification being accomplished by recrystallization from ethyl ether to afford a mixture of 2 and 3 (230 mg).

12-Methoxy-9(10→20)-abeo-*16,17-bis*-nor-*abieta-8',10' (20), 11,13-tetraen-7-one* (1) oil $[\alpha]_D^{23}$-59,6 (c, 10.0, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O), 1640 (C=C), 1595, 1495 (C=C skeletal), 1250, 1140 (C-O). EIMS m/a rel.int.): 284 (92, M^{+}), 269 (17, M-·CH_3), 256 (39, M-CO), 241 (28, M-CO-CH_3) or M-CH_3-CO), 216 (36, 1a + H·), 215 (88, 1a), 202 (100,1b), 201 (22, 1b-H.), 187 (22, 1a-CO), 185 (44, 1a-CH_2O), 173 (92, 1c), 171 (23,1b-OMe), 141 (30), 130(20), 129(36), 128(25), 115(36). ^{1}H and ^{13}C NMR: Table 1. UVλ $^{EtOH}_{max}$ nm (log ε), 255 (2.47), 260(2.84), 293 (0.48), 336 nm (0.31).

Mixture of 3β-O-cinnamoyl (2) and 3β-O-dihydrocinnamoyl-lupeol (3) M.p. 210-212°C (ethyl ether). IR $\nu^{K\text{-}Br}_{max}$.cm^{-1} 1712 (C=O), 1629 (C=C), 1510 (arom), 1140, 1170(C-O). ^{13}C NMR, Table 2. ^{1}H HMR (300 MHz, $CDCl_3$,

TMS): δ 7.68 [d, J=16.5 Hz, H-7'(2), 7.6-7.5 [m, H-2', 6'(2)] 7.4-7.3 [m, H-3',5',(2)], 7.3-7.1 [m, H-2' to H-6'(3)], 6.45 [d,J=16.5 Hz, h-8'(2)]. 4.70[br.s,H-29a(2,3)], 4.63 [dd, J=10.3, J=4.5 HZ, H-3 (2)], 4.58 [br, s, H-29b (2,3)], 4.5 [dd, J=10.3, J=4.5 Hz, H-3 (3), 2.98 [t, J=7.8 Hz, -CH_2-7'(3)], 2.69 [t, J=7.8 Hz, CH_2 8'(3)], 2.40 [m, H-19 (2,3)], 1.69 [br,s,CH_3-30(2,3)], 1.38 to 0.7 [s, seven methyl group (2,3), EIMS m/z (rel.inte.): 558 [M^+ of 3 (absent)], 556 [M^+ of 2 (absent)], 409(1), 408(1), 205(9), 204(10), 203(17), 202(8), 201(10), 191(18), 190(23), 189(45), 188(9), 187(15), 175(20), 173(10), 163(10), 162(9), 159(12), 150(13), 149(12, 148(14), 147(32), 145(13), 137(16), 136(20), 135(46), 134(20), 133(37), 131(21), 123(26), 122(21), 121(59), 120(19), 119(39), 109(51), 108(30), 107(80), 106(19), 105(88), 104(27), 103(12), 99(16), 96(9), 95(81), 94(28), 93(90), 92(18), 91(100), 83(15), 82(12), 81(66), 80(13), 79(60), 78(13), 77(27), 71(16), 69(58), 68(64), 67(59), 65(10), 55(40).

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